INFLAMMATION AND PRECLINICAL AD: ASSOCIATIONS BETWEEN PERIPHERAL INFLAMMATORY BIOMARKERS, COGNITION, AND AMLYOID-β DEPOSITION IN NON-DEMENTED OLDER ADULTS

by

Lauren E. Oberlin

B.A. in Psychology and B.A. in Political Science, University of Connecticut, 2011

M.S. in Psychology, University of Pittsburgh, 2015

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This dissertation was presented

by

Lauren E. Oberlin

It was defended on

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and approved by

Howard Aizenstein, M.D., Ph.D., Professor, Department of Psychiatry

Stephen B. Manuck, Ph.D., Professor, Department of Psychology

Anna L. Marsland, Ph.D., Professor, Department of Psychology

Beth Snitz, Ph.D., Associate Professor, Department of Neurology

Aidan G. C. Wright, Ph.D., Assistant Professor, Department of Psychology

Dissertation Director: Kirk I. Erickson, Ph.D., Professor, Department of Psychology

INFLAMMATION AND PRECLINICAL AD: ASSOCIATIONS BETWEEN PERIPHERAL INFLAMMATORY BIOMARKERS, COGNITION, AND AMLYOID-β DEPOSITION IN NON-DEMENTED OLDER ADULTS

Lauren E. Oberlin, Ph.D.

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The central role of inflammatory processes in the development of beta-amyloid (Aβ) pathology has been widely shown in rodent models, but has yet to be elucidated in humans, particularly prior to the onset of clinical symptoms. We examined associations between peripheral inflammatory mediators, cognition, and two neuroimaging Alzheimer's disease (AD) biomarkers, Aβ plaques and hippocampal atrophy, in non-demented older adults. Cross-sectional and longitudinal data were used to assess associations between peripheral inflammatory biomarkers (soluble CD14, TNF-α receptor concentrations, and IL-6) and memory performance on the California Verbal Learning Test (CVLT) and Rey-Osterrieth Complex Figure Test in 173 non-demented older adults. Of these 173 participants, 134 were cognitively normal (CN) and 34 had MCI. Ninety of these participants underwent repeated assessments at 24 months. Structural MRI and Pittsburgh compound B-PET imaging were used to quantify hippocampal volume and Aβ plaque deposition. After adjusting for demographics, linear regression analysis revealed that higher levels of TNF-α and IL-6 predicted poorer global and verbal memory performance in the full sample, and the CN subsample. Elevated concentrations of pro-inflammatory markers were also associated with higher global Aβ deposition, specifically among those that also exhibited greater hippocampal atrophy. Secondary analysis using template-derived regions of interest showed that these moderation effects were specific to PiB uptake in the anterior cingulate gyrus, frontal cortex, and precuneus. These associations remained after adjusting for hypertension, diabetes, heart disease and white matter

lesions (all $p<0.05$). Furthermore, higher levels of circulating IL-6 predicted subsequent conversion to MCI and increased longitudinal accumulation of Aβ pathology in regions susceptible to early amyloid deposition. Hippocampal volume moderated the association between inflammatory markers and Aβ deposition, suggesting potential disease-state-dependent differences in peripheral inflammatory profiles during the preclinical phase of AD. These findings highlight potential protein signatures that may vary depending on the prodromal phase of disease progression, and could help identify those in specific preclinical stages. from CN to MCI. Moreover, chronic, low-level systemic inflammation may accelerate the deposition of Aβ pathology and, consequently, place individuals at a higher risk of developing clinically significant cognitive impairment.

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PREFACE

To my Dad, Gary Oberlin, for your kindness, your warmth, your resilience, and your pure, unadulterated love of science; you are the alpha to my omega. To my Mom, Kate King-Oberlin, for your love, your strength, your intellect, and your ambition; I hope someday to become a fraction of the woman you are. To my sister, for being a constant source of admiration and love, I am and will always be inspired by you. And to my other sisters, Chelsey, Emily, Kate, and Kristen, I will forever be grateful for the steadfast love and support you have given me. Finally, my heartfelt thanks to my graduate advisor, Kirk Erickson. Your guidance, wisdom, and unwavering support has been invaluable; thank you for making this possible.

1.0 INTRODUCTION

Alzheimer's disease (AD) is the 6th leading cause of death in the United States (Association, 2013), with nearly 46 million currently suffering from AD or a related dementia worldwide (Dá Mesquita et al., 2016). Given the anticipated expansion of the aging population, the number of AD cases is expected to reach 100 million by the year 2050 (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007; Gupta & Iadecola, 2015). AD is a progressive brain disorder characterized by early impairments in learning and memory, followed by more severe declines in cognition, mood, and motor abilities and eventually the inability to complete even simple tasks (Alzheimer's Association, 2013Association (2013). These symptoms are preceded by neurodegenerative changes that begin at least 10-15 years prior to behavioral symptoms. In fact, one of the neuropathological hallmarks of Alzheimer's disease, amyloid beta (Aβ), is evident in the brain more than a decade prior to the onset of memory symptoms (Counts, Ikonomovic, Mercado, Vega, & Mufson, 2016; Mortamais et al., 2016; R. Sperling, Mormino, & Johnson, 2014). This extended preclinical period likely represents a *critical window* in which interventions may delay or prevent the onset of clinical symptoms.

At present, AD is incurable. Given the growing health (Brookmeyer et al., 2007; Gupta & Iadecola, 2015), psychosocial, and economic burdens (Alzheimer's Association, 2013) associated with AD, establishing approaches for slowing or preventing disease development is a public health imperative. The purpose of the proposed study is to assess factors that may exacerbate AD pathogenesis *early* in the disease course in order to facilitate early detection of AD and inform future efforts to delay or prevent disease progression.

Neuroinflammation is one mechanism that may contribute to the pathogenesis of Aβ early in the disease course (Canter, Penney, & Tsai, 2016; Heneka et al., 2015). Inflammation in the brain is generally an adaptive response to injury in which pro-inflammatory cytokines help reduce injury and eliminate destroyed tissue (Dá Mesquita et al., 2016). However, in AD, perturbations in the inflammatory response occur, which can exacerbate neural injury and cell death (Bronzuoli, Iacomino, Steardo, & Scuderi, 2016; Dá Mesquita et al., 2016) and may perpetuate or accelerate Aβ pathology. Evidence of this comes from rodent models in which sustained neuroinflammatory processes promote the synthesis and deposition of neurotoxic Aβ species (Decourt, Lahiri, & Sabbagh, 2016; Liao, Wang, Cheng, Kuo, & Wolfe, 2004; Sheng et al., 2003). Further, introducing an anti-inflammatory agent minimizes Aβ generation and neuronal loss and improves learning and memory deficits in rodent and primate models (Decourt et al., 2016; He, Cheng, Staufenbiel, Li, & Shen, 2013; Tweedie et al., 2012). While these results implicate neuroinflammation in the formation and accumulation of Aβ, translational work in humans is limited by a lack of both longitudinal studies and studies conducted during the presymptomatic stage of AD. Consequently, the impact of inflammatory processes on the pathogenesis and progression of Aβ early in the disease course is not well understood. An enhanced understanding of the mechanisms that perpetuate the neurodegenerative processes in AD, particularly prior to the onset of clinical symptoms, is imperative to inform the development of targeted interventions aimed at delaying or even preventing disease progression. The proposed study aimed to explore the relationship between inflammatory factors and preclinical AD pathology, and to assess the contribution of peripheral markers of inflammation to the longitudinal progression of Aβ.

To this end, I leveraged data from an ongoing imaging sub-study of an NIH funded multi-site placebo-controlled double-blind clinical trial of daily Gingko biloba (Ginkgo Evaluation of

Memory Study (GEMS). Following the completion of this multi-site clinical trial in 2008, 173 non-demented older adults (mean age = 86) from the Pittsburgh site were recruited to participate in the GEMS Imaging Sub-Study. All participants completed annual neuropsychological assessments. At baseline, 139 participants were cognitively normal and 34 were classified as having Mild Cognitive Impairment (MCI)(Lopez et al., 2014). Subjects provided blood samples from which serum levels of peripheral inflammatory markers were quantified, including concentrations of pro-inflammatory cytokines Interleukin-6 (IL-6), soluble levels of TNF-α receptor 1 (TNF-αRI), and TNF-α receptor 2 (TNF-αRII), and cell-surface receptor CD14. Fibrillar Aβ plaque deposition was measured *in vivo* using PiB-Positron Emission Tomography (PET) imaging approximately 10 months after the initial blood draw and once again two years later. Individuals in the preclinical phase of AD are well represented in this sample, as 48% of this cohort exhibited Aβ pathology at baseline, which increased to 75% at follow-up (Hughes, Kuller, et al., 2014), making this sample uniquely suited to explore both the cross-sectional and longitudinal relationships between inflammatory load at baseline and Aβ burden.

Using these data, I addressed the following aims:

Aim 1: Assess the relationship between peripheral inflammatory markers and cognitive status at baseline.

H1: Higher concentrations of pro-inflammatory markers will be associated with increased risk of meeting clinical criteria for MCI.

H2: Higher levels of peripheral inflammatory markers will be associated with reduced memory performance. A composite measure of memory will be calculated reflecting each participants combined performance on visual memory (Rey-Osterrieth Complex Figure Test (R-O)) and verbal memory (California Verbal Learning Test (CVLT) tasks.

Aim 2: Investigate the associations between baseline inflammatory markers and fibrillar Aβ deposition, using continuous measures of PiB-PET retention. In addition, assess whether the relationship between inflammatory markers and Aβ deposition varies as a function of preclinical disease stage.

H1: Raised levels of inflammatory markers will be associated with elevated global PiB retention at baseline.

H2: The magnitude of the positive association between peripheral inflammatory markers and PiB retention will be larger among individuals exhibiting greater neurodegeneration (i.e., hippocampal atrophy) at baseline. This relationship will be observed in the combined sample (MCI and CN), and in the asymptomatic subsample (e.g., excluding those with MCI).

Aim 3: Assess the relationship between peripheral inflammatory markers and change in cognitive status (i.e., conversion to MCI/dementia) over a two-year period.

H1: Elevated levels of pro-inflammatory markers at baseline will predict an increased risk of conversion to MCI at the 2-year follow-up.

H2: Higher concentrations of peripheral inflammatory markers will be associated with a decline in memory performance from baseline to follow-up.

Aim 4: Investigate whether peripheral inflammatory markers predict the progression of Aβ deposition over 2 years.

H1: Elevated levels of inflammatory markers will be associated with greater Aβ deposition at follow-up, as well as increased accumulation of Aβ between baseline and follow-up assessments, in the combined sample as well as subjects considered CN at baseline.

H2: This relationship will be moderated by preclinical disease stage, such that the positive relationship between inflammatory markers and change in Aβ deposition over the 2-year follow-up will be greater among those that also exhibit neurodegeneration at baseline.

1.1 BACKGROUND: ALZHEIMER'S DISEASE

Alois Alzheimer identified the first case of AD in 1901, and over the past century Alzheimer's disease has come to represent one of the greatest public health challenges worldwide. In fact, both the World Alzheimer's Report and the 2013 United States Alzheimer's Association Report indicate that, without the discovery of effective prevention and treatment measures, the number of AD cases may increase 2-3 fold by 2050 (Alzheimer's Association, 2010). The current annual cost of this disorder is estimated at $203 billion in the United States alone and is expected to grow alongside the increased prevalence of AD in the upcoming decades. Despite numerous and varied clinical trials, extant medications only provide temporary symptomatic relief for a subset of patients (Bronzuoli et al., 2016), and thus, a worldwide effort is currently under way to establish treatments to stop, slow, or prevent AD (Bronzuoli et al., 2016). Notably, according to the Alzheimer's Association, developing an intervention that would delay the onset of AD by 5 years would result in a 45% reduction in the number of AD cases by 2050 and decrease the estimated Medicare costs from $627 billion to $344 billion (Alzheimer's Association, 2010).

AD is the most common cause of dementia and accounts for an estimated 50-60% of all cases of dementia (Association, 2014). While short-term and episodic memory problems are often the first cognitive symptoms observed in AD, early signs may vary from person to person and can also include visuospatial deficits and difficulties with word-finding (Tarawneh & Holtzman,

2012). As the disease progresses, cognitive symptoms worsen and extend to other domains including impairments in judgment, reasoning, language, processing speed, and attention. Over time, these impairments begin to interfere with the ability to independently complete instrumental activities of daily living (e.g., getting dressed, cooking, managing medications and finances). Along with cognitive decline, changes in mood and personality, including apathy, aggression, and depression, are common in AD. In the final stage of the disease, even simple physical functions are impaired and individuals are completely dependent on others for their care. Age is the greatest risk factor for AD, as estimates indicate the vast majority of those with AD (82%) are 75 years or older (Alzheimer's Association, 2014Association, 2014). Although the duration of illness is variable, on average, individuals survive for 4 to 8 years after the initial diagnosis of AD (Association, 2013, 2014). Thus, AD is a pervasive, incurable condition characterized by progressive changes in cognition, mood, and physical function, that imparts considerable burdens on those that it afflicts as well as their loved ones.

1.2 NEUROPATHOLOGY OF ALZHEIMER'S DISEASE

Alzheimer's disease is characterized in the brain by two pathogenic features, extracellular senile Aβ plaques and neurofibrillary tangles (NFTs) (Heppner, Ransohoff, & Becher, 2015). Senile plaques are comprised of insoluble aggregates of Aβ protein fragments, which form after amyloidogenic cleaving of the amyloid precursor protein (APP). NFTs are composed of large deposits of hyperphosphorylated tau, a protein that, in a healthy brain, helps to maintain the structural integrity of axonal microtubules (Rubio-Perez & Morillas-Ruiz, 2012). A definitive diagnosis of AD requires the observation of both fibrillar Aβ plaques and NFTs in post-mortem

analysis (Karran, Mercken, & De Strooper, 2011). While it is understood that Aβ plaques and NFTs are centrally involved in initiating and promoting the synaptotoxic and neurodegenerative changes that underlie the onset and progression of AD, the etiology of AD remains poorly understood. In fact, along with Aβ plaques and NFTs, it is now widely recognized that the pathogenesis of AD likely involves a complex network of processes involving various cell types that interact to provoke a cycle of cellular dysfunction, injury, and death (Musiek & Holtzman, 2015). However, the particular role of other cellular and molecular process, such as oxidative stress and neuroinflammation, that may initiate or exacerbate these hallmark features of AD are not well understood.

The most widely accepted model of AD etiology, known as the amyloid cascade hypothesis, suggests that Aβ deposition plays an early and critical role in initiating many complex pathologic changes that lead to cell death, synaptic dysfunction, and eventual symptom manifestation. Briefly, Aβ peptides result from the aberrant cleavage of the amyloid precursor protein (APP). In the non-amyloidogenic pathway (i.e., in a healthy brain), APP is sequentially cleaved first by the α-secretase enzyme followed by γ-secretase (Heppner et al., 2015). For reasons that are not entirely understood, in the amyloidogenic pathway, APP is initially cleaved by the β-secretase complex (Canter et al., 2016; Dá Mesquita et al., 2016; Murphy & LeVine III, 2010). The subsequent cleavage of APP by γ-secretase generates Aβ peptides of varying lengths, including neurotoxic Aβ species ($A\beta_{42}$), which are prone to aggregation (Heppner et al., 2015; Oh, Madison, Villeneuve, Markley, & Jagust, 2013). These go on to form various Aβ aggregation states including Aβ oligomers and fibrillar Aβ plaques (Canter et al., 2016; Dá Mesquita et al., 2016; Murphy & LeVine III, 2010). Furthermore, two major enzymes largely responsible for the clearance of Aβ from the brain, neprilysin and insulin degrading enzyme, are decreased in disease-

affected regions, so a significant amount of Aβ remains un-degraded (Dá Mesquita et al., 2016; Murphy & LeVine III, 2010). Thus, a sequence of molecular processes underlies the formation of amyloid plaques, however, a number of questions regarding this pivotal process remain. For instance, there is ongoing debate about whether abnormal processing versus dysfunctional clearance of Aβ peptides (or a combination of both) represents the primary etiological event in sporadic AD (Heppner et al., 2015). Furthermore, the precipitating events that trigger Aβ accumulation and the particular factors that exacerbate or accelerate Aβ deposition are poorly understood and require further investigation.

Aβ is largely believed to have an important, causative role in the pathogenesis of AD by mediating numerous downstream pathologies that lead to synaptic dysfunction and cell death. In a seminal study by Shankar, et al., (2008) Aβ protein oligomers were extracted post-mortem from AD brains and introduced into mouse hippocampi (Shankar et al., 2008). Results showed that introduction of soluble Aβ oligomers reduced dendritic spine density, inhibited long-term potentiation, and facilitated long-term depression. Based on these findings, the authors concluded that the pathogenic effects of Aβ in AD include subtle alterations in synaptic structure and function (Shankar et al., 2008). Along with directly impacting synaptic processes, Aβ accumulation also leads to oxidative damage and impaired regulation of intracellular calcium levels, which can impair cellular functioning and lead to cell death (Canter et al., 2016). Furthermore, through various processes that are not fully understood, Aβ accumulation and deposition promotes the hyperphosporylation of tau proteins (Kantarci et al., 2012) and exacerbates the spread of NFTs (Musiek & Holtzman, 2015). Specifically, *in vitro* and *in vivo* studies in rodent models indicate that, at the molecular level, Aβ may promote tau pathology by stimulating specific tau-targeting kinases or by inducing proteases that may modify tau (Musiek & Holtzman, 2015). A recent study

also found that Aβ plaques facilitated the spread of NFT's from the initial site of aggregation in the mesial temporal lobe into the broader neocortical areas, and concluded that pathological tau formation and transmission requires the Aβ plaque environment (Jacobs et al., 2018). Tau hyperphosphorylation results in a number of neurodegenerative changes that lead to symptom manifestation, including the disassembly of microtubules, impaired axonal transport, and retrograde degeneration and cell death. Thus, Aβ alone does not directly cause all of the pathologic changes seen in AD, however, it is critical in the processes of initiating and enhancing numerous downstream pathologies that subsequently lead to symptom onset and disease progression.

1.3 AMYLOID PATHOLOGY AND THE PRECLINICAL PHASE OF AD

AD is characterized by a protracted preclinical phase, in which neuropathological processes are initiated many years, if not several decades, before the onset of clinically detectable cognitive symptoms (Racine et al., 2016). Aβ is the earliest detectable pathological change in AD (Jansen et al., 2015; R. A. Sperling et al., 2011), with estimates suggesting that Aβ deposition begins 10-20 years before the expression of cognitive and behavioral symptoms. Thus, the National Institute on Aging and the Alzheimer's Association (NIA-AA) have updated their diagnostic guidelines to include three phases of disease progression, the first of which is a preclinical phase in which clinical symptoms are absent but the neuropathological processes of AD are underway (Association, 2011; Counts et al., 2016). Moreover, within this preclinical stage, the NIA-AA have proposed a 3-stage chronological model of neuropathological progression beginning with 1) initial Aβ accumulation without contemporaneous neuronal injury, then 2) Aβ pathology *and* downstream changes in the form of neurodegeneration and synaptic dysfunction (e.g.,

hippocampal atrophy, cellular hypometabolism, tau pathology), followed by 3) amyloidosis, neurodegeneration, and the onset of subtle cognitive changes that do not yet meet criteria for MCI (Counts et al., 2016; Jack et al., 2011; R. Sperling et al., 2014). Consistent with this model, human studies largely show that Aβ aggregation precedes markers of neurodegeneration, tau pathology, and cellular hypometabolism, which occur prior to the onset of subtle cognitive deficits (Counts et al., 2016). This preclinical staging framework is not intended to be used in clinical settings. Instead, the NIA-AA believes this framework will offer researchers the opportunity to identify and investigate AD at the earliest possible point, and thereby aid in the development of treatment approaches that can be introduced prior to the onset of clinical symptoms (Association, 2011; Jack et al., 2011). In sum, Aβ deposits, brain atrophy, and tau pathology are apparent prior to the onset of clinically significant cognitive decline, with amyloid pathology representing the first detectable neuropathological change in this degenerative cascade.

The initial preclinical stage of AD is followed by mild cognitive impairment (MCI) (Minor Neurocognitive Disorder-DSM-V), which is characterized by noticeable, mild to moderate declines in cognitive functioning that exceed normal, age-related changes but do not interfere with the ability to function independently in daily life (Association, 2011). The third and final stage of disease progression is dementia due to Alzheimer's disease, which involves a substantial decline from a previous level of performance in memory and at least one other cognitive domain that impairs independence in everyday activities (Association, 2011). Large-scale pharmaceutical trials aimed at slowing or reversing AD have generally been conducted during these latter two stages, after cognitive and behavioral symptoms have clinically manifested (Canter et al., 2016; Musiek & Holtzman, 2015; R. A. Sperling et al., 2011). Despite the evidence supporting Aβ pathology as an early and critical initiator of downstream neurodegenerative changes, trials targeting Aβ have

failed to yield clinical improvements or halt further cognitive decline (Canter et al., 2016; R. A. Sperling et al., 2011). Even trials that observed reductions in Aβ burden based on biomarker or autopsy analysis did not find meaningful clinical improvements (Dá Mesquita et al., 2016). Thus, pharmaceutical interventions introduced during this phase of disease development show very little benefit. These unsuccessful outcomes have been linked to the fact that cellular dysfunction and neurodegenerative changes are well-entrenched by the time cognitive and behavioral symptoms are clinically detected. Indeed, studies in cognitively unimpaired older adults have shown that diffuse, global Aβ deposition throughout the cortex, as well as high mesial temporal levels of tau pathology are apparent prior to the onset of cognitive deficits (Villemange, 2018). In order to delay or prevent further neurodegeneration and the onset of clinical symptoms, numerous researchers have suggested that the greatest chance for success may lie in targeting these pathological changes at the earliest possible moment in the disease course, before Aβ-related downstream changes have begun to take place (Dá Mesquita et al., 2016; Musiek & Holtzman, 2015; R. A. Sperling et al., 2011; R. A. Sperling, Karlawish, & Johnson, 2013). Thus, the preclinical stage of AD represents a critical window in which targeting the underlying brain changes *early* in the disease course may delay or prevent the onset of clinical symptoms. However, much remains to be known about the pathophysiologic mechanisms that initiate and enhance Aβ accumulation, particularly early in the disease course before symptoms have become clinically manifest.

One of the greatest barriers to understanding, diagnosing, and treating the preclinical phase of AD is the absence of observable clinical symptoms. Therefore, the NIA-AA developed a working criteria of fluid and imaging biomarkers that can be used to evaluate the presence of AD pathology in this critical, asymptomatic phase (Counts et al., 2016; Sharma & Singh, 2016). Specifically, Stage 1 of the preclinical phase, characterized as isolated Aβ pathology, can be

measured in the cerebrospinal fluid or using PET imaging. The NIA-AA recommends that biomarker evidence of neurodegeneration, reflecting Stage 2 of the preclinical phase, be defined as either hippocampal atrophy (using Magnetic Resonance Imaging), abnormal cellular metabolism (i.e., hypometabolism) on 18F-fluorodeoxyglucose (FDG)-PET, or elevated tau pathology. Aβ deposition and tau pathology can also be measured in humans *in vivo* with PET imaging, using radiotracers capable of binding to tau or fibrillar Aβ plaques. [11C]-Pittsburgh Compound B (PiB) is a radiotracer that preferentially binds to fibrillar Aβ species and can quantify insoluble Aβ deposits in the brain. Standard uptake value ratios (SUVRs) are calculated that represent tracer retention, with positive tracer uptake indicative of fibrillar Aβ deposition. Whole brain or global amyloid burden is traditionally estimated and dichotomized into Aβ/PiB positive (Aβ+) or Aβ/PiB negative (Aβ-) using threshold-based approaches thought to reflect pathologically significant levels of Aβ accumulation. Assays for monomers of Aβ measured in the CSF can also provide indirect estimates of circulating concentrations of $A\beta_{42}$ and the ratio of $A\beta_{42}/A\beta_{40}$. Of note, however, CSF-derived estimates of amyloid pathology are limited by a lack of regional specificity, as well as observed temporal lags between *in vivo* detection of Aβ burden using PET imaging and CSF-measured Aβ, suggesting a limited ability for CSF to reflect the neural environment (Gordon, 2018). This staging framework is illustrated in Figure 1.

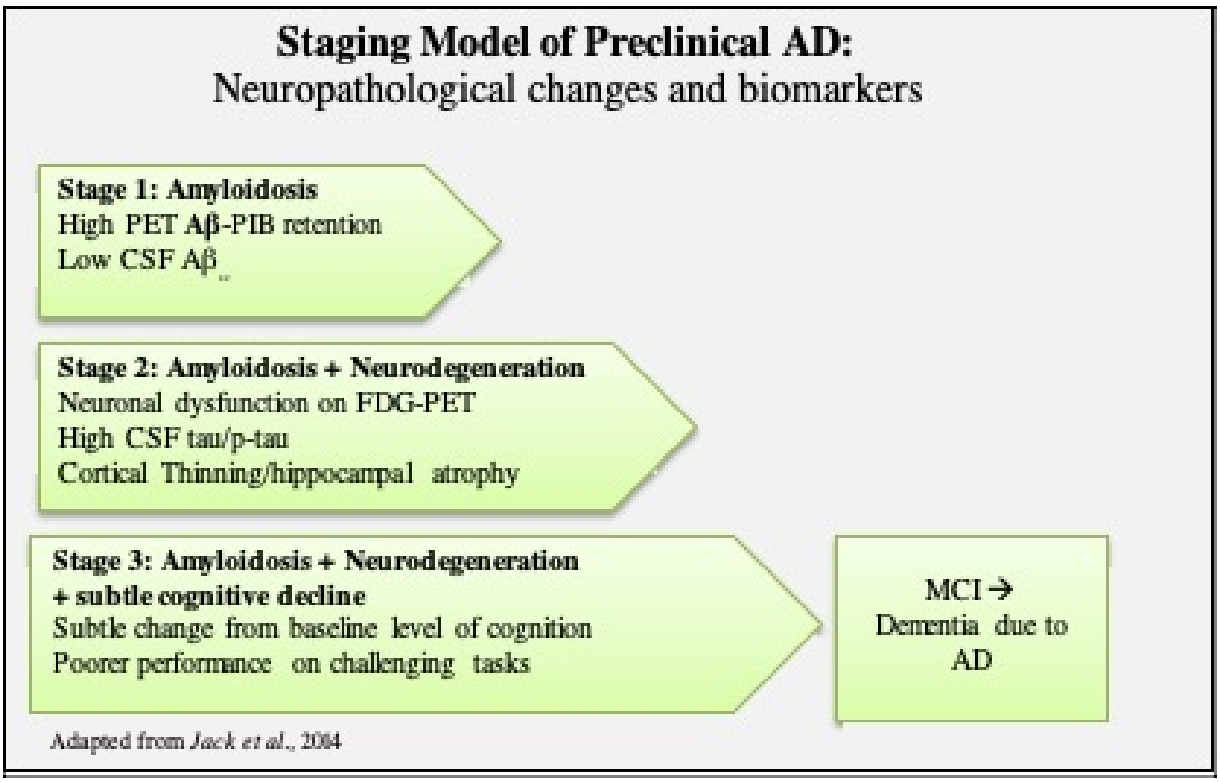

Figure 1. Staging model of preclinical AD.

The advent of CSF and imaging markers of Aβ have aided in early detection of AD pathology, and have allowed researchers to investigate the pathophysiological and cognitive correlates of preclinical AD and disease progression. The hypothesized chronology of preclinical disease progression, beginning with isolated Aβ accumulation followed by subsequent markers of neurodegeneration (hippocampal atrophy, cerebral hypometabolism), has been confirmed using these biomarkers in recent longitudinal studies (Jack et al., 2014; Vos et al., 2013). For instance, in a large prospective epidemiologic study of preclinical AD, Jack et al., (2014) found that rates of amyloid accumulation were independent of baseline hippocampal atrophy, while atrophy rates were dependent on the degree of initial amyloid load, with elevated amyloid burden predicting accelerated atrophy rates in cognitively normal older adults (Jack et al., 2014). These studies have also revealed the regionally-specific evolution of these pathological processes. Specifically, AD-

related cell death and tau deposition share proximal and temporal specificity, and are initially apparent in the medial temporal regions followed by subsequent spreading into the medial and lateral regions of the cerebral cortex (Cho et al., 2016; He et al., Villemange et al., 2018). Aβ aggregation occurs prior to these degenerative changes, and staging models of early amyloid deposition reveal a distinct, regional pattern of pathological accumulation. Although there is some variability across studies, Aβ deposits tend to develop first and most substantially in the medial frontal cortex and precuneus, then the lateral frontal, inferior temporal, and parietal lobes, followed by progression to the occipital and sensory-motor areas, and finally the medial temporal lobe and striatum (Cho et al., 2016; (Vlassenko, Benzinger, & Morris, 2012);(Grothe et al., 2017); (Villeneuve & Jagust, 2015)). Thus, the frontal cortex and precuneus are among the regions most vulnerable to early amyloid accumulation, with posterior and subcortical structures relatively spared until later in the preclinical phase.

1.4 AMYLOID, COGNITION, AND THE BRAIN

1.4.1 Aβ pathology in cognitively normal older adults

Amyloid pathology is not only apparent in individuals with MCI and AD, but also among a proportion of older adults clinically considered to be cognitively healthy. Although there is some variability across studies in the prevalence of Aβ pathology present in cognitively normal older adults, estimates generally suggest that between 20-40% of CN adults aged 60-90 have elevated levels of Aβ pathology in the brain (Lim, Maruff, et al., 2013; Oh et al., 2013; R. Sperling et al., 2014; R. A. Sperling et al., 2011). Of note, the cognitive prognosis for those exhibiting Aβ

pathology is worse than those without significant levels of Aβ deposition (Chételat et al., 2013). Longitudinal evidence suggests that CN individuals with biomarker evidence of amyloid plaques (CSF, blood biomarkers or PET imaging) are 4 times more likely to develop clinically significant cognitive impairment/dementia within the subsequent 2-7 years relative to those with little to no amyloid pathology (Musiek & Holtzman, 2015). For example, in a longitudinal study of 206 older adults (average age ~ 73), Villemagne et al., (2011) found that elevated baseline levels of PiB tracer retention were observed in 97% of participants with dementia of the Alzheimer's type, 69% of subjects with MCI, and 31% of healthy controls. Furthermore, 25% of healthy controls with elevated PiB uptake at baseline progressed to MCI or dementia of the Alzheimer's type by 36 months. In contrast, only one subject in the PiB- group (1.4%) had developed MCI at the time of follow-up (Villemagne et al., 2011). Thus, evidence suggests that the presence of amyloid pathology is associated with an increased risk of developing MCI or dementia, although the extent to which Aβ predicts the clinical course and subsequent onset of dementia is not well understood. In fact, some studies have included participants that manifested the pathological symptoms of AD but never expressed clinical symptoms of impairment in their lifetime (R. A. Sperling et al., 2011). However, it is unclear whether these participants would have developed clinical symptoms had they lived longer (R. A. Sperling et al., 2011). Thus, the presence of Aβ pathology is not yet considered a deterministic marker of future AD; however, it is strongly predictive of subsequent risk for cognitive decline and disease development.

Although Aβ burden is associated with future risk of MCI and dementia of the AD type, whether Aβ deposition is associated with more subtle deficits in cognitive performance in CN older adults remains a matter of debate. Using CSF and brain imaging biomarkers of Aβ burden, some studies have observed an inverse association between Aβ pathology and cognitive

performance among CN adults (Kantarci et al., 2012; Lim, Ellis, et al., 2013; Petersen et al., 2016; Rentz et al., 2010), while other work has failed to detect this relationship (Aizenstein et al., 2008; Marchant et al., 2013; Mormino et al., 2014; Storandt, Mintun, Head, & Morris, 2009). In one of the largest prospective studies to assess this to date (n = 286 older adults), Petersen et al., (2016) found that the 31.7% of cognitively healthy adults exhibiting elevated Aβ levels at baseline demonstrated poorer cognitive performance in all domains assessed aside from language, including measures of executive function, attention, memory, and visuospatial processing, relative to PiB- participants, as well as reduced hippocampal volume and lower glucose metabolism. Furthermore, elevated PiB uptake at baseline was associated with an accelerated rate of annual cognitive decline in all domains aside from language, as well as a greater annual increase in ventricular volume and hippocampal atrophy relative to PiB- subjects (Petersen et al., 2016). These results suggest that in CN older adults, elevated Aβ deposition might be associated with subtle cognitive deficits in a broad range of cognitive domains as well as regionally specific brain atrophy and hypometabolism. To provide a quantitative summary of this literature, a recent meta-analysis examined the associations between Aβ burden and cognitive performance across 34 studies, which represented data from approximately 3,485 CN older adults (Hedden, Oh, Younger, & Patel, 2013). Across all studies, Aβ pathology (measured with imaging or fluid biomarkers) was significantly associated with reduced performance on measures of episodic memory, executive function, and global function while measures of working memory, processing speed, semantic memory, and visuospatial function were not associated with Aβ burden (Hedden et al., 2013). In sum, a growing body of evidence suggests that Aβ deposition is associated with subclinical changes in cognitive function. However, the presence and magnitude of the association between Aβ pathology and cognition varies across studies, and the domain specificity of this relationship

is poorly understood. The incongruous findings across studies may be attributable to small sample sizes, variations in the type and sensitivity of the cognitive measures, the parameters used to define CN adults (Insel et al., 2016), and variations in subject demographics and disease progression across studies.

These discrepancies may also be a consequence of the approaches used to estimate and quantify amyloid pathology. Specifically, while some studies incorporated continuous measures of amyloid pathology, others dichotomized participants into groups of PiB+ and PiB- using conventional stratification thresholds. However, recent work suggests that amyloid positivity, traditionally defined using global signal thresholds, may correspond to relatively advanced stages of preclinical amyloid deposition (Villeneuve et al., 2017; Grothe et al., 2017; Jansen et al., 2018). For instance, a recent study in 665 CN participants found that those considered to be in the earliest stages of amyloidosis (e.g., regionally distinct tracer retention in the medial frontal, lateral parietal and temporal lobes) were classified as amyloid negative using standard binary cutoffs (Grothe et al., 2017). Consistent with this, another recent study in 154 CN older adults found that global measures of amyloid burden and conventional dichotomization approaches lacked the sensitivity to detect regionally restricted amyloid accumulation that occurs in the earliest phases of amyloid progression (Villeneuve et al., 2017). The authors argued that the hierarchical progression of amyloid accumulation that takes place in the earliest phases of amyloid pathogenesis may be eclipsed, or go undetected, by using global estimates. For instance, a large prospective sample of cognitively normal older adults found that elevated levels of Aβ deposition among subjects classified as amyloid negative using standard thresholds was associated with poorer cognitive trajectories over 4 years (Landea et al., 2018). Furthermore, in a separate sample of CN older adults categorized as Aβ- negative using conventional criteria, elevated Aβ deposition significantly

predicted subsequent tau deposition in regions associated with AD over the course of 5 years (Leal et al., 2018). The authors emphasized the importance of identifying and targeting these populations before Aβ is widespread throughout the cortex, and underscored the need for future studies to apply less conservative signal thresholds and incorporate continuous measures of global and regional PiB retention to better elucidate these relationships. Notably, ROI-based approaches have recently been applied to evaluate the relationship between early regional Aβ aggregation and other neuropathological markers associated with AD, including tau pathology (Lockhart et al., 2017) and hippocampal atrophy (Sone et al., 2017).

1.4.2 Moderators of the Aβ-cognition relationship

Genetic, behavioral, and other factors may also moderate the relationship between amyloid burden and cognitive function in CN populations. For example, mounting evidence suggests that carriers of the APOE ε4 genotype, which represents a major genetic risk factor for sporadic AD, have higher rates of Aβ pathology and exhibit accelerated amyloid plaque accumulation relative to similarly-aged non-carriers (Petersen et al., 2016). For instance, in a sample of 144 CN older adults, Lim and colleagues found that greater cerebral Aβ burden was associated with poorer performance on visuospatial and episodic memory tasks. Furthermore, when the sample was stratified by APOE status, the relationship between Aβ pathology and cognition was only observed in APOE ε4 carriers (Lim, Ellis, et al., 2013). Kantarci et al., (2012) also found that the magnitude of the Aβ-cognition relationship was greater among APOE ε4 carriers, even after carriers and non-carriers were matched on cortical PiB retention (Kantarci et al., 2012). Cognitive reserve may also moderate this relationship, as Rentz et al., (2010) found that the inverse relationship between Aβ binding and cognition was mitigated among individuals with greater years of education and a

higher premorbid verbal IQ (Rentz et al., 2010). Recent work also suggests that higher fitness levels are associated with reduced Aβ deposition (B. Brown et al., 2013; Head et al., 2012) and may moderate Aβ-related effects on cognition (Schultz et al., 2017).

Finally, the relationship between Aβ and cognition may also depend on where individuals fall on the continuum of preclinical AD (e.g., whether other downstream neurodegenerative processes have begun to take place). For instance, Morimono et al., (2014) assessed the independent and combined impact of Aβ plaque deposition and neurodegeneration on cognitive performance in 166 CN older adults. Fibrillar Aβ deposition was assessed using PiB-PET imaging, and neurodegeneration was estimated using measures of hippocampal volume and regionally specific hypometabolism using FDG-PET (Mormino et al., 2014). Measures of hypometabolism and hippocampal volume were used to approximate different phases of neuropathological changes, with subjects exhibiting elevated Aβ *and* hypometabolism/hippocampal atrophy considered further along the preclinical continuum (e.g., phase 2). While Aβ pathology and neurodegeneration were independently associated with reduced performance on cognitive tasks, the combination of both neurological features was associated with even poorer performance on cognitive tasks and a greater magnitude of decline over the course of two years (Mormino et al., 2014). These findings are consistent with the staging framework outlined by the NIA-AA, and are supported by recent longitudinal work showing that the subsequent appearance of neurodegeneration (i.e., hippocampal atrophy, cerebral hypometabolism) following initial Aβ aggregation underlies the onset or increases the magnitude of subtle cognitive changes (Jack et al., 2014; Vos et al., 2013 (Aschenbrenner, Gordon, Benzinger, Morris, & Hassenstab, 2018)). Thus, various factors may moderate the relationship between Aβ pathology and cognitive function, including the combined presence of neurotoxic Aβ species and downstream neurodegenerative changes.

1.4.3 Summary of Aβ, cognition, and AD

In sum, AD is an increasingly prevalent and incurable form of dementia that is pathologically defined by hyperphosphorylated tau tangles and the deposition of fibrillar Aβ plaques. One major challenge for treatment is the protracted preclinical phase of AD, in which neurodegenerative processes begin many years or several decades before the onset of clinically detectable cognitive symptoms (Counts et al., 2016; Mortamais et al., 2016; R. Sperling et al., 2014). The aggregation of abnormally cleaved Aβ species commences 10-20 years before clinical symptoms arise, and initiates numerous downstream changes that drive the neurodegenerative process in AD. While pharmaceutical trials targeting the generation and accumulation of Aβ have shown limited benefit, these have almost exclusively been conducted in samples with overt clinical symptoms (MCI or AD)(Canter et al., 2016; Dá Mesquita et al., 2016; R. A. Sperling et al., 2011). Targeting AD pathology at the earliest possible phase, by minimizing Aβ aggregation and progression, could delay or prevent subsequent neurodegeneration and even symptom manifestation. However, to successfully forestall the progression of Aβ pathogenesis, it is imperative to obtain an enhanced understanding of the cellular and molecular processes that initiate and exacerbate Aβ early in the disease course.

1.5 NEUROINFLAMMATION AND AD PATHOGENESIS

1.5.1 Overview of neuroinflammatory processes under pathological conditions

Inflammatory processes are initiated early in the course of disease onset, and may play a critical role in the pathogenesis and progression of AD. The inflammatory response in the brain is important to eliminate the initial cause of cell injury, repair damaged tissue, and remove necrotic cells (Calsolaro & Edison, 2016; Rubio-Perez & Morillas-Ruiz, 2012). Neurons, astrocytes, and microglia are responsible for the inflammatory reaction in the brain (Rubio-Perez & Morillas-Ruiz, 2012). Microglia, which comprise 10% of the cells in the central nervous system (CNS), are considered the resident macrophages of the CNS as they act as the first line of defense against neuronal injury and invading pathogens (Heppner et al., 2015; Walters, Phillips, Zheng, Biju, & Kuruvilla, 2016; W.-Y. Wang, Tan, Yu, & Tan, 2015). Under homeostatic conditions, microglia act as surveyors, searching for cell damage and pathogens, removing apoptotic neurons and helping to maintain synapses (Heneka et al., 2015; Krstic & Knuesel, 2013). Under pathological conditions, like brain injury, altered neuronal function, or the detection of pathogens, microglia become activated (Heneka et al., 2015; W.-Y. Wang et al., 2015). In order to fight off an injury/infection as quickly as possible, activated microglia initiate an immune response and interact with astrocytes and neurons to minimize damage to the healthy brain (Heneka et al., 2015; Ramesh, MacLean, & Philipp, 2013). Activated microglia have a number of functions including phagocytosis, tissue repair, and mediating the production of chemokines, cell adhesion molecules, trophic factors and pro and anti-inflammatory cytokines (Heneka et al., 2015; Walters et al., 2016; W.-Y. Wang et al., 2015). Cytokines are a diverse class of small proteins that are secreted by microglia and astrocytes and act as signaling molecules that help to regulate the duration and

intensity of the immune response (Rubio-Perez & Morillas-Ruiz, 2012). Cytokine signaling can result in a diverse cascade of events that regulate inflammation and modulate cellular functions, including T-cell differentiation, apoptosis, and phagocytosis (Ramesh et al., 2013; Rubio-Perez & Morillas-Ruiz, 2012). Cytokines can be pro-inflammatory or anti-inflammatory (i.e., cytokines that suppress genes for pro-inflammatory cytokines), and can promote the further activation of microglial cells and the production of other cytokines (Rubio-Perez, 2012). Thus, microglia act as critical mediators of the immune response in the brain, in part, by initiating the production of signaling molecules (cytokines) that help to regulate the inflammatory drive.

When an acute injury occurs, this rapid response by the innate immune system is meant to be protective, helping to resolve the pathological changes with immediate benefit to the local neural environment (Calsolaro & Edison, 2016; Fan, Okello, Brooks, & Edison, 2015; Heneka et al., 2015). However, if tissue health is not restored and the pathogenic stimulus persists, a chronic state of inflammation unfolds (Calsolaro & Edison, 2016; Heneka et al., 2015; Rubio-Perez & Morillas-Ruiz, 2012). Repeated or sustained exposure to inflammatory conditions leads to microglial priming, in which the response of microglia to inflammatory signals becomes sensitized or exaggerated (Krstic & Knuesel, 2013; Walters et al., 2016). This phenomena is illustrated in rodent models, which have shown that chronic or repeated peripheral inflammatory challenge primes microglia to remain in an activated state or induce an exaggerated inflammatory response in the face of subsequent stimulation (Heneka et al., 2015; Krstic & Knuesel, 2013).This primed migroglial phenotype commonly occurs in neurodegenerative conditions and, to a lesser magnitude, in healthy aging (Heneka et al., 2015). Microglial priming results in an amplified or prolonged inflammatory response, stimulating the further release of pro-inflammatory chemokines, cytokines, and reactive oxygen species, which ultimately contributes to and expands

cellular dysfunction, neuronal injury and death (Heneka et al., 2015). Thus, while the response of the innate immune system is initially neuroprotective, a phenotypic shift occurs in microglial cells in the context of sustained inflammatory conditions that exacerbates the degenerative process.

1.5.2 Mechanisms linking neuroinflammation and Aβ pathology: evidence from rodent and primate models

Rodent models demonstrate that the early and progressive aggregation of neurotoxic Aβ peptides initiates a prolonged inflammatory response that, in turn, exacerbates neural injury and may promote disease progression. Pathological changes within all brain tissues are sensed by immune cells, including microglia, which are capable of detecting pathogens and significant alterations in cellular functioning (Heppner et al., 2015). In murine models of AD, the production of Aβ stimulates an innate immune response, beginning with the binding of microglial cells to Aβ species via cell-surface receptors, including CD14, CD36, and CD47 (Heneka et al., 2015). Activated microglia then stimulate the production of pro-inflammatory chemokines and cytokines, including TNF-α, IL-6, and IL-1α, which have numerous downstream effects including further activation of microglial cells and phagocytosis (Calsolaro & Edison, 2016; W.-Y. Wang et al., 2015). The persistent accumulation of Aβ leads to a primed microglial state (Heneka et al., 2015; Krstic & Knuesel, 2013; Walters et al., 2016), typified by an enhanced sensitivity to subsequent inflammatory stimuli and a sustained production of pro-inflammatory signaling molecules. Consistent with this, a greater concentration of Aβ in transgenic mouse models is associated with increased concentrations of pro-inflammatory mediators including TNF-α, IL-1α, and IL-6 (Heneka et al., 2015; Rubio-Perez & Morillas-Ruiz, 2012). The role of Aβ as a key initiator of the inflammatory response in AD is highlighted in a recent rodent study by Balducci and colleagues

(2017), which found that a single injection of Aβ oligomers into the lateral ventricle led to the rapid activation of glial cells and increased pro-inflammatory cytokine expression (Balducci et al., 2017). Indeed, disruption of the APP gene, which is a critical precursor to Aβ generation, delays and reduces microglial activation (Rubio-Perez & Morillas-Ruiz, 2012). The cellular changes that take place in the context of chronic inflammation have numerous downstream effects, including the production of reactive oxygen species and nitric oxide synthase, which promote cell dysfunction and exacerbate neural injury (Calsolaro & Edison, 2016; Heppner et al., 2015; Rubio-Perez & Morillas-Ruiz, 2012). Further, while activated microglia may initially promote Aβ clearance, hyperactive or primed microglia are less capable of clearing and degrading Aβ pathology, creating a cytotoxic environment for surrounding neurons and further promoting the aggregation of Aβ plaques (Hickman, Allison, & El Khoury, 2008). Taken together, this evidence indicates that, through numerous mechanisms, Aβ species stimulate and perpetuate a heightened inflammatory response through microglial priming, which furthers the neurodegenerative process.

Subsequent studies have sought to determine whether these inflammatory processes are epiphenomenal or play a causal role in disease onset and progression. Evidence from rodent and primate models indicate a potentially bi-directional relationship between neuroinflammation and Aβ such that Aβ aggregates promote pro-inflammatory processes which, in turn, drive the accumulation and progression of neurotoxic Aβ species. For instance, reactive astrocytes and pro-inflammatory cytokines like TNF-α promote the up-regulation of the β-secretase cleaving enzyme, which facilitates the formation of toxic Aβ species (Calsolaro & Edison, 2016; Walters et al., 2016). Furthermore, Aβ pathology appears to increase under inflammatory conditions. In a seminal study by Liao et al., (2004), introduction of pro-inflammatory cytokines TNF-α and IL-1β increased β-secretase activity and initiated the production of intracellular Aβ. Similarly, using a

mouse model in which the precursor protein for Aβ was expressed, Guo (2002) and colleagues found that transgenic mice did not develop Aβ deposits under non-inflammatory conditions. However, inducing a systemic acute-phase response resulted in Aβ deposition that was preceded by an increase in cytokine concentrations in the brain (Guo, Yu, Grass, de Beer, & Kindy, 2002). The contribution of pro-inflammatory processes to Aβ progression has also been illustrated in rhesus monkeys. Leung et al., (2013) found that microglia isolated from the cortex of aged monkeys generated considerable reactive oxygen species when stimulated by fibrillar Aβ. Furthermore, delivery of a microglia inhibitory factor abolished fibrillar Aβ toxicity and led to a reduction in the volume of damage caused by Aβ (Leung et al., 2011). Similarly, blocking TNF-α signaling reduces the presence of Aβ plaques in transgenic mouse models (W.-Y. Wang et al., 2015). Furthermore, genetic deletion *in vitro* of microglial cell surface receptors CD26, TLR4, and TLR6, which facilitate microglial binding to Aβ species and subsequent activation, reduces cytokine production and also prevents the accumulation of Aβ (Heneka et al., 2015). In sum, pro-inflammatory cytokines, as well as cell-surface receptors that promote a pro-inflammatory cascade, enhance Aβ formation and aggregation. Rodent models further suggest that inhibiting the inflammatory response may minimize or eradicate Aβ progression (Heneka et al., 2015; Leung et al., 2011; E. Wang et al., 2016). These studies indicate a vicious cycle of disease progression in which microglial priming results from chronic exposure to Aβ pathology, leading to a sustained inflammatory response that exacerbates and heightens Aβ accumulation, thereby furthering the pro-inflammatory drive (Calsolaro & Edison, 2016; Heppner et al., 2015; Rubio-Perez & Morillas-Ruiz, 2012; W.-Y. Wang et al., 2015).

1.5.3 Mechanisms linking peripheral immune health to Aβ pathology

The above studies illustrate the influence of neuroinflammatory signaling on AD-related pathology, however, the impact of immune processes is not restricted to cells operating directly in the CNS. In fact, several lines of evidence reveal that the peripheral immune environment is capable of having neuromodulatory effects. While it was once believed that inflammatory cells in the circulation could not invade the CNS, it is now understood that complex, dynamic, and elaborate pathways exist that allow for peripheral immune cells to infiltrate the CNS and influence inflammatory processes in the brain. These pathways, reviewed by Capuron and Miller (2011), include the direct passage of cytokines through permeable regions of the blood brain barrier and the active transport of pro-inflammatory cells from the systemic circulation via transport molecules located on endothelial cells (Capuron & Miller, 2011). Notably, the pro-inflammatory response of brain-resident immune cells to AD pathology facilitates the activate transportation of inflammatory mediators from the systemic circulation. Specifically, Aβ deposits impact immune process by interacting with endothelial cells at the blood brain barrier. Indeed, introduction of fibrillary $A\beta_{42}$ has been found to induce the release of inflammatory cytokines from microglial and endothelial cells, including TNF-α, IL-6, and IL-1β (Griffin, Kho, Graham, Nicholson, & O'Carroll, 2016; W.-Y. Wang et al., 2015). These cytokines impair the integrity of the BBB, causing the BBB to become "leaky" and enabling the infiltration of peripheral immune cells including T cells and cytokines into the CNS (Heppner, 2015, Rezai-Zadeh 2009; MacPherson et al., 2017(Calsolaro & Edison, 2016; W.-Y. Wang et al., 2015). Once in the brain, peripheral cells interact with microglia and astrocytes, enhancing microglial priming and leading to the further release of cytokines and chemokines (Krstic & Knuesel, 2013; W.-Y. Wang et al., 2015). Moreover, rodent models show that prolonged exposure to Aβ deposits also results in the expression of local signaling molecules

(chemokines) in the CNS, which promote migration of cells from the periphery to the sites of Aβ aggregates in the brain (Dá Mesquita et al., 2016; Heneka et al., 2015). Thus, the neuroinflammatory response to Aβ deposition includes the expression of signaling molecules and pro-inflammatory mediators that modulate the permeability of the BBB and facilitate the migration of peripheral immune cells into the brain.

1.5.3.1 Peripheral immune health and Aβ pathology: evidence from rodent models

The capacity for the peripheral inflammatory environment to impact the CNS is illustrated by murine studies showing how perturbations in the peripheral immune environment lead to alterations in inflammatory signaling and Aβ deposition in the brain. For instance, initiating a targeted pro-inflammatory response in the *periphery* in APP transgenic mice has been shown to accelerate microglial activation and cytokine signaling in the CNS and increase Aβ plaque formation (Heneka et al., 2015; Krstic & Knuesel, 2013). For example, to stimulate a localized inflammatory response in the periphery, Kyrkanides and colleagues (2011) induced osteoarthritis in the knees and joints of an APP transgenic mouse model of AD. Mice with osteoarthritis exhibited elevated mRNA expression of a number of cytokines in the CNS, including IL-1β and TNF-α, as well as a greater number of Aβ plaques relative to mice without arthritis. Examining these rodent models over multiple time points also revealed that mice with osteoarthritis developed Aβ plaques sooner and more rapidly compared to controls, suggesting that an isolated systemic inflammatory event leads to an up-regulation of neuroinflammatory processes, and promotes and accelerates Aβ pathogenesis (Kyrkanides et al., 2011). Furthermore, MacPherson et al., (2017), found that *peripheral* administration of a TNF-α inhibitor in AD transgenic mice resulted in decreased brain populations of both centrally and peripherally derived immune

cells, including signaling molecules that facilitate the migration of peripheral cells into the CNS. Peripheral TNF-α inhibition also resulted in decreased Aβ density and cytokine expression in the hippocampus, and protected against deficits in long-term potentiation (MacPherson et al., 2017). Thus, peripheral inflammatory cells are capable of penetrating the BBB and reaching the brain, where they interact with inflammatory cells in the CNS to exacerbate the inflammatory drive and potentiate Aβ pathogenesis.

1.6 INFLAMMATION AND AD IN HUMANS

The current body of rodent work provides abundant and strong mechanistic support for the central and causal role of inflammatory processes in Aβ pathogenesis. Whether these findings are representative of the contribution of systemic inflammatory processes to AD onset and progression in humans remains poorly understood. However, studies have sought to address these gaps by evaluating the cognitive correlates of inflammatory expression profiles among those with AD, and have recently begun to explore the mechanisms underlying this relationship.

1.6.1 Inflammation, cognitive impairment, and AD

While the potentially pathogenic role of inflammatory processes in AD remains poorly understood, neuroinflammation is now widely recognized as a naturally occurring and predominant feature of nearly all neurodegenerative diseases, including AD (Heneka, 2015). The association between inflammatory factors and AD in humans was initially identified in post-mortem studies, which found that levels of pro-inflammatory cytokines including IL-6, were heightened in the brain tissue

of AD patients (Calsolaro & Edison, 2016; Strauss et al., 1992). More recent post-mortem work has shown regional co-localization between concentrations of microglia, astrocytes, and Aβ plaque deposition in AD, indicating a proximal and potentially functional relationship between Aβ pathology and inflammatory mediators (Heppner et al., 2015).

The correlation between immune processes and AD is further illustrated by studies conducted *in vivo,* which demonstrate heightened CSF and serum/plasma concentrations of pro-inflammatory cytokines and chemokines in participants with AD compared to CN control subjects. This is highlighted by a recent meta-analysis of 175 studies, which found significantly higher concentrations of an array of 14 pro-inflammatory cytokines, including IL-6 and TNF-α, among subjects with AD relative to CN controls (Lai et al., 2017). Another quantitative synthesis demonstrated that heightened concentrations of CRP and IL-6 predicted the subsequent onset of all-cause dementia (Darweesh et al., 2018). These patterns have been consistently observed in populations with advanced/severe AD (Brosseron et al., 2014). However, studies exploring variations in inflammatory profiles between cognitively healthy subjects and those with MCI or less advanced AD have yielded contradictory findings. For example, a meta-analysis of 22 studies failed to find group differences across a range of inflammatory biomarkers, including concentrations of cytokines, chemokines, and cell adhesion molecules, between MCI and CN control subjects (Saleem et al., 2015). Results from a large systematic review were in agreement, noting that while the majority of studies show elevated levels of pro-inflammatory mediators among those with MCI, collectively this body of literature was inconclusive (Brosseron et al., 2014). Nevertheless, it is unlikely that inflammatory biomarkers are exclusively elevated during advanced stages of cognitive impairment given established associations between systemic indicators of inflammation and cognitive performance in cognitively healthy populations. For

instance, a meta-analysis of prospective longitudinal studies of CN older adults found that elevated IL-6 levels predict subsequent cognitive decline, such that those with high baseline concentrations of IL-6 were 1.42 times more likely to exhibit global declines in cognitive performance over follow-up periods ranging from 2-7 years (Bradburn, Sarginson, & Murgatroyd, 2018). Taken together, this data suggests that a heightened inflammatory load is apparent in AD and may also be associated with the progression of clinical symptoms. However, as many of these authors note, the association between inflammatory markers and cognition in AD remains to be more fully elucidated, particularly earlier in the disease course. It is also unclear from this work whether inflammatory expression profiles represent a correlate of disease progression or an early phenomenon driving neurodegenerative changes.

These inconsistent findings may be a consequence of study-specific differences in sample demographics, biomarker specificity, and diagnostic criteria for MCI and dementia. Also underlying these disparate findings in non-demented samples may be disease-state-dependent differences in cytokine expression (Swardfager, et al., 2010), as inflammatory processes may play a pathological role *prior* to the onset of cognitive symptoms. Consequently, comparing biomarker concentrations between those with and without detectable cognitive impairment naturally overlooks those in the preclinical, asymptomatic phase that may already be exhibiting elevations in inflammatory signaling. Indeed, in their systematic review, Brosseron and colleagues (2014) note that the nature and specificity of cytokine expression may vary depending on the stage of disease progression. This is supported by a handful of studies that indicate that biomarkers may peak at distinct time points of disease development. For instance, a prospective longitudinal study failed to find differences in serum levels of TNF-α between those with AD, MCI, and CN subjects. However, those who progressed from MCI to AD exhibited elevated TNF-α concentrations

relative to those whose MCI status remained stable over time (e.g., did not progress to AD) (Diniz et al., 2010). Relatedly, another longitudinal study by Galimberti (2012) found that participants with MCI and mild AD exhibited elevated levels of a chemokine, monocyte chemotactic protein-1 (MCP-1) relative to controls, while group differences were not observed among those with severe AD (Galimberti et al., 2006). Furthermore, MCP-1 levels decreased over the course of the 1-year follow-up in the subgroup of subjects with MCI that converted to AD, leading authors to conclude that up-regulation of MCP-1 may be an early event in AD that far precedes the onset of clinical symptoms (Galimberti, Fenoglio, & Scarpini, 2008). However, the association between inflammatory markers and subtle cognitive deficits in the preclinical phase has been infrequently explored. Furthermore, whether these biomarkers predict progression to MCI in asymptomatic, at-risk individuals exhibiting AD-related pathology is not well understood. Determining how these associate with AD-related pathology prior to the onset of cognitive symptoms could help identify surrogate biomarkers or protein signatures for earlier disease detection and intervention.

1.6.2 Neuroinflammation and Aβ in humans: neuroimaging

Mounting evidence in humans indicates that subjects with AD exhibit a distinct pro-inflammatory profile that differs from CN controls, although when over the course of disease progression differences in biomarker expression begin to manifest remains a matter of debate. To expand upon this work and explicate the mechanistic underpinnings of this relationship in humans, recent studies have investigated associations between neuroinflammatory processes and AD-related pathology, including Aβ. This has been investigated in only a small handful of studies *in vivo* using PET imaging, which have used radiotracers that bind to activated microglia and astrocytes to measure inflammatory signaling in the CNS. In a sample of 20 older adults, Santillo et al., (2011)

observed higher microglial tracer uptake in the frontal, parietal, and medial temporal lobes among AD patients relative to CN controls, as well as a significant correlation between the binding potential of microglial tracers and PiB retention among the 9 subjects with AD (Santillo et al., 2011). This is consistent with findings from Fan et al, (2015), which also observed a correlation between the voxelwise distribution of microglial activation and the regional specificity of Aβ deposition (Fan et al., 2015). In one of the only studies comparing AD (n = 7), MCI (n = 7), and CN (n = 10) populations, Yasuno et al., (2012) found higher microglial uptake in both AD and MCI groups relative to CN controls, but failed to find a difference in microglial binding between MCI and AD groups. This may be attributable to the small sample size, but may also indicate a disease-state-dependent relationship such that microglial activation is enhanced early on in the disease course but reaches a plateau as the disease progresses (Yasuno et al., 2012). Notably, however, other small-scale studies have failed to detect any group differences in microglia/astrocyte activation between AD, MCI, and CN populations (Schuitemaker et al., 2013; Wiley et al., 2009).

These inconsistent findings may, in part, be due to methodological differences and measurement limitations across existing studies. Specifically, current microglia tracers are unable to isolate particular microglia or identify their specific mode of action (Fan et al., 2015). Given that activated microglia can exist in a range of phenotypic states, these tracers are unable to discriminate between microglial cells expressing a pro- versus anti-inflammatory phenotype. The lack of specificity of this approach limits interpretation, as it is unclear based on these data whether increased microglial activation (i.e., greater tracer uptake) reflects a reparative process, a pro-inflammatory/neurotoxic process, or a combination of protective and destructive processes. Therefore, the existing data offers a very circumscribed understanding of the role of the specific

cellular components of the inflammatory process in the pathogenesis of AD. Identifying particular inflammatory mediators associated with Aβ onset or progression may provide a starting point for the development of molecules able to modify specific cellular cascades. Finally, due to the nearly exclusive use of cross-sectional designs and the absence of studies conducted in preclinical populations, the extent to which inflammatory processes contribute to Aβ progression early in the disease course remains unknown.

1.6.3 Peripheral inflammation and Aβ pathology in humans

1.6.3.1 Distinguishing fluid biomarkers of immune cell expression

While the aforementioned studies in humans have employed PET imaging to broadly assess microglia/astrocyte activity in the brain, the study described in this thesis examined plasma concentrations of 3 specific inflammatory biomarkers: IL-6, TNF-α and CD14. While the use of blood-based biomarkers has superior specificity relative to PET imaging in that it is capable of detecting levels of particular proteins and cytokines of interest, unlike PET imaging, plasma data reflects concentrations of biomarkers in the peripheral circulation. At present, peripheral inflammatory markers are not considered to be directly representative of intracerebral concentrations (Woodcock & Morganti-Kossmann, 2013). Fluid markers of both amyloid pathology and inflammatory concentrations can also be measured in the CSF. Briefly, CSF is partly generated from the interstitial fluid of the brain, and because it is not separated from the CNS by the BBB, CSF has more contact with the brain than the peripheral circulation. Therefore, CSF-derived measures are considered the best obtainable fluid approximation of cellular processes in the brain, with blood biomarkers often compared to CSF-based markers to assess how well systemic measures correspond with or reflect the neural environment.

Currently, the extent to which blood measures of inflammatory markers represent or serve as a proxy for inflammatory processes in the CNS in humans is not well understood. One study found that plasma concentrations of IL-6 correlated highly with CSF-derived IL-6 levels in patients with AD (Sue et al., 2003), which has been shown more recently in healthy populations (Agorastos et al., 2014). However, other studies in non-demented samples have failed to find significant associations between the expression profiles of inflammatory markers measured in the CSF and plasma/serum (Lindqvist et al., 2009; Sasayama et al., 2013; Tsirpanlis et al., 2009). Thus, the extent to which peripheral inflammatory signaling captures or represents global inflammatory processes versus an independent immune state is not well understood. Nevertheless, the neuromodulatory impact of peripheral immune processes has been well established in rodent models, and studies in older adults illustrate the salient, and potentially distinct relationship between the systemic inflammatory environment and brain and cognitive heath.

1.6.3.2 Associations between peripheral inflammatory mediators, cognition and brain health in non-demented populations

Evidence of the relationship between the central and peripheral inflammatory environments in humans comes, in part, from observational studies showing that conditions or events that are restricted to the brain correlate with changes in peripheral cytokine expression (Bradburn et al., 2018). Specifically, blood levels of pro-inflammatory markers are heightened in multiple sclerosis (Macchi et al., 2015) and AD (Swardfager et al., 2010), have been positively associated with cerebral microbleeds (Miwa et al., 2011), and are elevated following traumatic brain injury (Ramlackhansingh et al., 2011; Woodcock & Morganti-Kossmann, 2013). While interpretation is limited by the use of cross-sectional data and the possibility of bidirectional effects (e.g., elevated

systemic inflammation may confer greater risk), this does indicate that the peripheral immune environment may serve as a biomarker of inflammatory events in the brain.

Prospective and epidemiological studies in older adult populations also reveal the sensitivity of the aging brain to peripheral immune challenge. For instance, heightened inflammatory signaling in the circulation following surgical interventions is one of the leading mechanisms thought to underlie post-operative cognitive dysfunction, an increasingly understood phenomena often observed in older adults that undergo surgeries outside of the CNS. In a meta-analysis of 54 studies, patients with post-operative cognitive dysfunction and post-operative delirium expressed significantly higher peripheral concentrations of two markers of systemic inflammation, CRP and IL-6, relative to those that were cognitively healthy following surgery (Liu, Yu, & Zhu, 2018). These group differences were not observed pre-operatively, suggesting that the systemic inflammatory response to surgical intervention resulted in an up-regulation of peripheral immune cells that corresponded to an increased risk of cognitive impairment (Liu et al., 2018). Furthermore, one small prospective study in 56 individuals with MCI and 25 healthy controls found that greater CSF concentrations of TNF-α predicted conversion from MCI to dementia over a 9-month period (Tarkowski, Andreasen, Tarkowski, & Blennow, 2003).

Neuroimaging studies in cognitively healthy older populations provide further evidence of the link between systemic inflammation and brain health. For instance, in a sample of cognitively healthy older adults, Zhang et al., (2016) found associations between TNF-α and IL-6 and reduced grey matter volume in the occipital temporal cortex, bilateral medial PFC, and inferior parietal lobule (Zhang et al., 2016). These associations remained even after adjusting for demographic factors, APOE ε4, geriatric depression, and cardiovascular risk factors. Plasma/serum measures of IL-6 have also been linked with reduced hippocampal volume (Marsland et al., 2015), and another

marker of systemic inflammation, CRP, has been associated with cortical thinning in non-demented older adults (Carlier et al., 2018). Taken together, whether peripheral biomarkers provide an indirect or partial representation of the CNS milieu, or capture distinct elements of peripheral immune health is not well understood. However, the aforementioned studies in cognitively healthy aging populations illustrate the relationship between cytokine signaling in the periphery and brain structure and function.

1.6.3.3 Associations between peripheral inflammation, cognition, and brain health in AD

The sensitivity of the aging brain to the influence of systemic inflammation may be exacerbated in early AD. It is well understand that communication between immune cells in the periphery and the CNS is not only possible, but more likely in pathological states, such as during the early accumulation of Aβ species. Accordingly, it has been hypothesized that the neuroinflammatory response to initial Aβ aggregation (e.g., microglial priming/sensitivity, upregulation of pro-inflammatory signaling, increased BBB permeability) may make the brain more vulnerable to a second immune challenge. This second immune challenge may include an acute event such as an infection, as well as chronic systemic inflammation (Eikelenboom et al., 2012; Yasuno et al., 2017). Consistent with this hypothesis, severe infections in the periphery among those with AD have been found to yield cognitive consequences. In a seminal prospective study of 300 older adults with AD, acute systemic inflammatory events (defined as a short lived (< 2 months) infection or trauma occurring outside of the CNS) were associated with elevated serum levels of TNF-α and a 2-fold increase in the rate of cognitive decline over the course of 6 months (Clive Holmes et al., 2009). Conversely, those who had low serum TNF-α levels throughout the study exhibited no cognitive decline over the course of 6-months. Thus, inflammatory events that occurred in the periphery resulted in heightened TNF-α levels and predicted a more rapid rate of

cognitive decline. These findings are consistent with this two-step hypothesis, which suggests that early Aβ aggregation results in a neuroinflammatory cascade that facilitates the ability for peripheral cells to infiltrate the CNS and enhances the influence of these immune cells on Aβ deposition. Along with acute illness, rodent models indicate that the systemic inflammatory environment may also act as a contributing factor towards further neuroinflammatory signaling and Aβ pathogenesis.

In humans, the role of the peripheral pro-inflammatory drive in AD onset is supported by mounting evidence showing that cardiovascular disease (CVD) risk factors that result in a sustained, pro-inflammatory state represent well-known risk factors for AD. Specifically, hypertension, midlife obesity, insulin resistance, and high cholesterol, all of which correlate with chronic, low-grade inflammation, are predictors of late-life AD ((Barnes & Yaffe, 2011; Calsolaro & Edison, 2016; Kivipelto et al., 2001; Kivipelto et al., 2005; Rethorst, Bernstein, & Trivedi, 2014; Srikanthan, Feyh, Visweshwar, Shapiro, & Sodhi, 2016; Welty, Alfaddagh, & Elajami, 2016; Whitmer, Sidney, Selby, Johnston, & Yaffe, 2005; Zammit, Katz, Derby, Bitzer, & Lipton, 2015). Notably, emerging work has also demonstrated a relationship between CVD risk factors and AD-related neuropathology. A series of recent studies have revealed associations between PET-derived measures of Aβ burden and genetic markers of cholesterol transport (Hughes, Lopez, et al., 2014), high blood pressure (Hughes, Lopez, et al., 2014), overweight/obesity (Glodzik et al., 2016), and elevated triglycerides (Choi et al., 2016). Using the GEMS sample of non-demented older adults, arterial stiffness, a proxy for atherosclerosis, was recently found to predict the progression of Aβ pathology over the course of two years (Hughes, Kuller, et al., 2014). A recent, population-based prospective study also demonstrated the cumulative effect of multiple cardiovascular risk factors on subsequent Aβ burden 20 years later (mean age at follow-up = 73) (Gottessman et al., 2017).

Thus, while the mechanisms linking CVD risk factors to AD risk and Aβ burden are not fully understood, systemic inflammation has been identified as one probable pathway underlying these associations. Specifically, the early deposition of Aβ may represent an initial inflammatory event that permits pro-inflammatory cells that proliferate in a state of chronic inflammation to have additive or even distinct neuromodulatory effects.

1.6.4 Circulating inflammatory biomarkers and AD-related pathology

To date, very few studies have evaluated the associations between CSF and blood indicators of inflammatory biomarkers and AD-related pathology. Magalhães and colleagues (2018) explored associations between systemic inflammatory markers, CSF-derived estimates of AD-related proteins (Aβ and tau), hippocampal volume and functional connectivity among CN older adults and those with amnestic MCI and mild AD. They failed to find associations between inflammatory biomarkers and CSF-measured $A\beta_{42}$. However, relative to cognitively healthy controls, individuals with amnestic MCI and mild AD exhibited elevated levels of serum cytokines TNF-α, IL-10, and IL-12, which corresponded to decreased functional connectivity in the default mode network, which is commonly disrupted in AD (Magalhães et al., 2018). Given that similar trends were observed in subjects with dementia and amnestic MCI, the authors concluded that systemic inflammation may correlate with functional connectivity disruption, even early in disease progression. Using a panel of 11 inflammatory and vascular biomarkers measured in the CSF, Janelidze et al., (2018) explored whether inflammatory mediators correlated with disease phase. Participants were categorized into preclinical (Aβ+), prodromal (Aβ+/MCI) or dementia groups based on neuropsychological data and CSF-derived estimates of Aβ and tau pathology. They found that cell adhesion molecules, but not CSF inflammatory cytokines (e.g., IL-6, IL-7, IL-10), were

elevated among those in the preclinical, prodromal, and dementia stages (Janelidze et al., 2018). Conversely, D'Anna et al., 2018 measured plasma levels of IL-6 and IL-10 in 18 CN patients and 27 with AD, and found that both pro-inflammatory biomarkers were associated with CSF-measured $A\beta_{42}$ among those with AD (D'Anna et al., 2017). Taken together, this circumscribed body of literature suggests potential associations between inflammatory mediators and AD-related pathology. However, the current work is limited by the use of small sample sizes, populations with symptomatic AD, and a reliance on CSF measures of inflammatory markers and Aβ pathology. Of note, CSF-based Aβ estimates may not be detectable until Aβ accumulation has reached global levels in the brain, making it harder to identify biomarkers that may be most temporally relevant or may peak at earlier phases of disease onset.

In fact, markers of systemic inflammation may represent independent immune processes in the periphery and provide information that is distinct from CSF-based immune measures. For instance, a recent meta-analysis of 54 studies assessed differences in blood and CSF obtained cytokine concentrations among individuals with AD relative to CN controls. Pooled estimates across 40 studies measuring *peripheral* cytokine levels showed that concentrations of pro-inflammatory cytokines including IL-6, TNF-α, TGF, IL-1, IL-12, and IL-18 were elevated in AD participants compared to cognitively healthy controls, while no group differences were observed in concentrations of interferon gamma (INF-γ), anti-inflammatory cytokines IL-4 or IL-10, pro-inflammatory cytokine IL-8, or C-reactive protein (CRP). Interestingly, among the 14 studies that measured cytokine concentrations in the CSF, there was not a significant difference in IL-6 or TNF-α between cognitively healthy and AD participants (Swardfager et al., 2010). These findings not only demonstrate the potential biomarker specificity of this relationship, they also suggest that inflammatory markers derived from the circulation may capture information that is unique from

that measured in the CSF. In the context of amyloid pathology, a recent study evaluated whether CSF and plasma measures independently predicted markers of AD pathology in a sample of non-demented middle-aged and older adults with a family history of AD. Bettcher and colleagues (2018) found that plasma concentrations of pro-inflammatory cytokine IL-8 were positively associated with CSF levels of amyloid burden (sAAPβ), while measures of the same biomarker obtained in the CSF showed no association with amyloid load (Bettcher et al., 2018). Furthermore, the relationship between plasma IL-8 and sAAPβ remained after adjusting for CSF IL-8, suggesting a potentially distinct pathway linking peripheral inflammation to amyloid pathology. Taken together, although these results require further exploration, they indicate that circulating inflammatory biomarkers may be uniquely or independently associated with brain structure and function in AD.

Only one study, to our knowledge, has examined the association between peripheral immune signaling and amyloid burden *in vivo* using PET imaging. Specifically, in a sample of 36 cognitively normal older adults aged 55-85, Yasuno and colleagues (2017) collected peripheral concentrations of a number of lymphocytes including B cells (CD19+, regulatory T cells (CD4+, FOXP3 +, CD25+), and cytotoxic T cells (CD8+ and CD3+). Subjects were categorized into low and high PiB-PET groups based on the global cortical mean signal uptake using conventional thresholds. They found the percentage of cytotoxic T cells (CD3+ and CD8+) to be significantly greater among subjects in the high PiB group relative to those in the low PiB group after controlling for age, gender, and education level. Including global PiB retention as a continuous variable revealed that the percentage of cytotoxic T cells in the periphery explained 17% of the variance in global amyloid burden, after adjusting for demographic factors. Given that this study was conducted in a non-demented sample, the authors concluded that peripheral immune changes start

early in disease onset, prior to the manifestation of cognitive deficits. They also note that these results lend support to the notion that inflammatory processes in the brain of individuals with Aβ pathology may have systemic parallels (Yasuno et al., 2017).

1.6.5 Anti-inflammatory treatments and AD

Given the compelling link between inflammation and AD pathogenesis, anti-inflammatory agents including NSAIDs and glucocorticoid steroids have been considered as treatment options for AD. Rodent models have shown that treatment of mice that overexpress APP with an anti-inflammatory agent (Ibuprofen) reduces microglial activation and the accumulation of Aβ plaques in the cortex (Yan et al., 2003). Furthermore, upon exposure to TNF-α, neurons that were pretreated with ibuprofen showed reduced production of Aβ relative to untreated neurons (Rubio-Perez & Morillas-Ruiz, 2012). The potentially protective effects of anti-inflammatory agents in humans were initially observed in epidemiological studies of populations with osteoarthritis or rheumatoid arthritis, who are conventionally treated with anti-inflammatory drugs such as NSAIDs for long periods of time. These findings, along with subsequent cross-sectional research, confirmed an inverse relationship between treatment with NSAIDS for conditions like arthritis and the incidence of AD (Heneka et al., 2015; Walters et al., 2016). In addition, a prospective, observational study found a reduction in AD risk among subjects who had regularly taken NSAIDs for at least 24 months (In'T Veld et al., 2001; Rubio-Perez & Morillas-Ruiz, 2012). While findings from animal models and observational epidemiological studies have shown promise, randomized, placebo-controlled, double-blind trials of anti-inflammatory treatments for AD have had little success (Heneka et al., 2015; Rubio-Perez & Morillas-Ruiz, 2012; Walters et al., 2016). Specifically, administration of NSAIDS for 8-16 months had no effect on the rate of cognitive decline or disease

progression in individuals with AD (Heneka et al., 2015). Similar to pharmaceutical trials targeting Aβ, disease-modifying interventions using anti-inflammatory agents have been largely conducted in populations with MCI or AD, and thus the timing of treatment initiation may be critical. Despite this, the temporal relationship between inflammatory processes and neuropathological progression, particularly during the preclinical stage of AD, has been remarkably understudied. In order to sufficiently clarify the role of inflammatory factors in AD, longitudinal studies in humans are necessary to disentangle the complex functions of these processes early in the course of disease onset.

1.6.6 Summary of existing literature

The aggregation of neurotoxic Aβ species is an early and critical event in the pathogenesis of AD, contributing to a cascade of neuropathological changes that result in synaptic and neural dysfunction, cell death, and eventual symptom manifestation. However, pharmaceutical trials targeting the generation and deposition of Aβ in populations with MCI or AD have been largely unsuccessful. Targeting Aβ earlier in the disease course, before downstream neurodegenerative changes have taken place, may be the most promising approach to modify disease progression. As described by Sperling et al., (2011), "it is possible that similar to cardiac disease and cancer treatment, AD would be optimally treated before significant cognitive impairment, in the presymptomatic or preclinical stages of AD" (R. A. Sperling et al., 2011). In order to effectively target Aβ, a thorough understanding of the mechanisms that perpetuate this pathogenic process is necessary, although currently, these are poorly understood.

Inflammatory processes may have a central and causal role in the pathogenesis of AD, as evidenced by numerous rodent and primate models. According to these studies, Aβ aggregation

initiates a prolonged inflammatory response, resulting in a cascade of events that exacerbates tissue damage and neurotoxicity, and may also promote the generation and progression of Aβ pathology. Administration of anti-inflammatory agents reduces Aβ burden in transgenic mouse models, and observational, population-based studies indicate that long-term use of NSAID's is associated with a reduced incidence of AD onset. However, randomized placebo-controlled pharmaceutical trials in AD populations have failed to generate clinical improvements or delay symptom progression. The preclinical phase may be an optimal time to initiate a therapeutic intervention, however, the implementation of such trials are hampered by a circumscribed understanding of the relationship between inflammatory factors and AD pathology early in the disease course. This is further complicated by the fact that the inflammatory cascade, at least initially, may involve a combination of neuroprotective and neurotoxic functions. Extant work in humans is limited by 1) the use of small sample sizes, 2) methodological approaches that are unable to differentiate between pro- and anti-inflammatory processes in activated cells 3) focused only on samples with clinically detectable cognitive impairment, and 4) have almost exclusively involved cross-sectional designs.

1.7 PRESENT STUDY

The present study aims to address these gaps in the literature by using a large sample of non-demented older adults with measures of specific peripheral inflammatory biomarkers and PiB-PET measures of Aβ deposition with longitudinal follow-up. Gaining a better understanding of the association between specific inflammatory biomarkers and the deposition and progression of Aβ plaques in preclinical AD will offer insight into whether regulating particular cells may modify

disease progression, and thus could inform the development of preventative treatments and help identify the optimal time to initiate targeted interventions.

We hypothesized that elevated concentrations of circulating pro-inflammatory biomarkers would be associated with poorer memory performance and greater global amyloid deposition at baseline. Given our use of an entirely non-demented sample, we further hypothesized that these biomarkers would predict amyloid burden specifically in brain regions susceptible to early Aβ deposition, including the anterior cingulate gyrus, lateral frontal cortex, and the precuneus. Given the plasticity of the microglial phenotype and the sensitivity of these cells to sustained inflammatory stimuli and early Aβ accumulation, it is possible that the influence of particular inflammatory mediators on Aβ deposition may vary as a function of disease phase. We used a theoretically driven approach to assess potential disease-state-dependent differences based on two converging bodies of literature: 1) rodent models indicating alterations in the inflammatory response to early Aβ species which, in turn, promotes Aβ pathogenesis and 2) the temporal sequencing of neuropathological changes in preclinical AD. Therefore, consistent with the preclinical staging framework outlined by the NIA-AA, hippocampal atrophy was used in the present study as a biomarker of neuropathological progression. By including hippocampal volume as a moderator, we sought to estimate preclinical disease phase by identifying those that may be exhibiting downstream neurodegenerative changes (corresponding to phase 2 of the preclinical staging framework). While there are limitations to this approach, which are elaborated on in the discussion, hippocampal volume has frequently been used in cross-sectional and longitudinal studies to distinguish individuals representing more advanced stages of preclinical disease progression. We hypothesized that the association between pro-inflammatory signaling and amyloid deposition would be greater in magnitude among those further along the preclinical

continuum (e.g., those exhibiting downstream neurodegenerative changes). Finally, we anticipated that elevated levels of systemic inflammatory mediators would predict cognitive decline and progression of amyloid pathology over the 2-year follow-up period.

2.0 METHODS

2.1 PARTICIPANTS

Participants were recruited from a multisite, double-masked, placebo controlled randomized clinical trial of daily use of Ginkgo biloba (GEMS trial) (Mathis et al., 2013). The GEMS study began in September 2000 and concluded with a final closeout visit between October 2007 and March 2008 (Hughes, Kuller, et al., 2014; Hughes, Lopez, et al., 2014; Lopez et al., 2014). The trial involved 3,069 community dwelling older adults aged 72 to 96 years at baseline, including 966 participants at the University of Pittsburgh site. The primary aim of the study was to assess whether daily use of 240 milligrams of Gingko biloba would reduce the incidence of all-cause dementia. Results indicated that daily Gingko biloba use did not slow or delay the development of dementia, and did not result in less cognitive decline in CN subjects or those with MCI (Snitz et al., 2009). A subsample of 194 non-demented subjects from the Pittsburgh site were subsequently recruited to participate in the GEMS Imaging Sub-Study, which is an ongoing longitudinal observational study involving annual or biannual visits that include neuroimaging and cognitive assessments.

Participants for the original GEMS study were recruited from 4 US communities including Sacramento, California (University of California-Davis), Winston-Salem and Greensboro, North Carolina (Wake Forest University), Pittsburgh, Pennsylvania (University of Pittsburgh), and Hagerstown, Maryland (Johns Hopkins University). Participants were recruited using voter registration and other purchased mailing lists (DeKosky et al., 2008).

2.1.1 Exclusionary criteria

Exclusionary criteria for the GEMS clinical trial included 1) baseline dementia 2) use of the anticoagulant warfarin 3) use of tricyclic antidepressants, antipsychotics, cholinesterase inhibitors, or other medications with psychotropic or cholinergic effects, 4) daily use of 400-IU of vitamin E, 5) electroconvulsive therapy within the past 10 years or hospitalization for depression within the past year, 6) history of bleeding disorders, 7) history of Parkinson's disease, 8) abnormal thyroid (serum creatine > 2.0 mg/d) or liver function tests at initial assessment, 9) hematocrit level < 30%, 10) low baseline vitamin B12 levels (< 210 pg/mL), 11) platelet count < 100 X $10^3/\mu$ L, 12) an unwillingness to discontinue taking over-the-counter Ginkgo biloba, or 13) a disease-related life expectancy of < 5 years (DeKosky et al., 2008; Snitz et al., 2009). Exclusionary criteria for the subsequent GEMS Imaging Sub-Study include dementia at the GEMS closeout visit (in 2008) or contraindications for neuroimaging (Mathis et al., 2013).

2.2 PROCEDURE

In the GEMS clinical trial (2000-2008), participants were randomized to receive either 240 mg daily of Gingko biloba extract or a placebo (median follow-up of 6.1 years) (DeKosky et al., 2008; Snitz et al., 2009). For the purposes of the present study, only data acquired at the final GEMS visit is relevant and therefore detailed here. Participants returned for a final GEMS closeout visit between October 2007 and March 2008. At this time, participants completed a neuropsychological assessment and resting blood pressure, height and weight data, and a blood draw were obtained (Hughes, Lopez, et al., 2014). Approximately 10 months after the GEMS closeout visit, the 194

participants that were recruited into the GEMS Imaging Sub-Study completed a neuropsychological assessment. Neuroimaging measures were also taken at this time, including brain MRI and PET using the Aβ radiotracer PiB. Two years later, in 2011, 183 participants returned for follow-up cognitive assessments and 103 underwent neuroimaging, including both MRI and PiB-PET imaging. Other studies using this dataset have found that participants in the GEMS Imaging Sub-Study were slightly younger than the full sample of 671 participants at the Pittsburgh site that completed the original GEMS protocol (84.0 [SD 2.8] versus 84.4 [SD 3]), but otherwise these samples did not differ in terms of gender, ethnicity, education, estimated premorbid intelligence, or APOE ε4 status (Mathis et al., 2013; Snitz et al., 2013). The 103 subjects in the GEMS Imaging Sub-Study that returned for follow-up imaging assessments in 2011 did not differ from the baseline sample of 183 subjects with respect to age, gender, APOE ε4 status, cognitive status in 2009 or body mass index (Hughes, Kuller, et al., 2014). The GEMS clinical trial and the GEMS Imaging Sub-Study were approved by the institutional review board and required participants to complete informed consent prior to any study procedures (Hughes, Kuller, et al., 2014; Lopez et al., 2014). The study timeline for the GEMS trial and the following GEMS Imaging Sub-Study can be found in Figure 2.

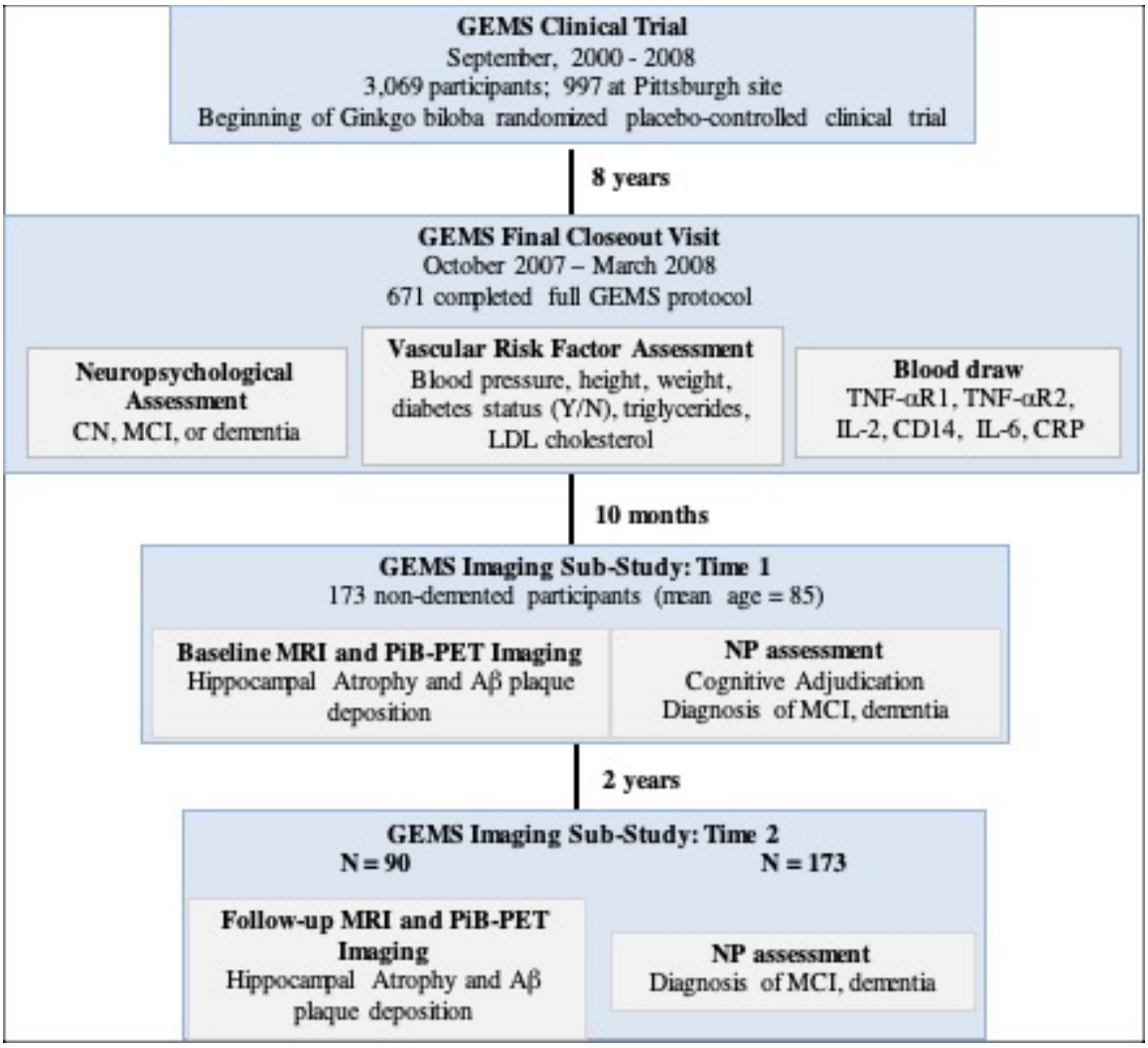

Figure 2. GEMS study timeline.

2.3 IMAGINE ACQUISITION AND PREPROCESSING

2.3.1 Magnetic Resonance Imaging

MRI data were collected using a GE Signa 1.5 T scanner and a standard head coil (Price et al., 2005). A high-resolution T1-weighted volumetric spoiled gradient recalled sequence (SPGR) was

acquired (0.937 X 0.937mm) in either the sagittal or coronal orientation with the following parameters: TE/TR = 5/25; flip angle = 40 degrees; slice thickness = 1.2mm/0mm interslice (Price et al., 2005).

The high-resolution structural data obtained from the MR sequence was used to estimate hippocampal volume. Specifically, hippocampal volume was calculated for each subject and normalized to each participants total intracranial volume (ICV). An automated approach was used to estimate total ICV, which reflects the volume within the "inner skull" and was computed using FMRIB's Brain Extraction Tool (Lopez et al., 2014). Hippocampal volume was calculated using an atlas-based segmentation approach. Hippocampal ROIs were defined using a reference brain (MNI) and normalized to fit each participant's anatomical image (Lopez et al., 2014). A ratio of total bilateral hippocampal volume relative to total ICV was calculated and multiplied by 100, which was used as a continuous estimate of hippocampal atrophy/neurodegeneration in statistical models. To create a binary variable indicative of neurodegeneration, a W-score was also calculated. This is a commonly used approach to assess and classify hippocampal atrophy that is consistent with an AD profile (Jack et al., 2008; Knopman et al., 2012; Prestia et al., 2013; Wirth et al., 2013). In the present study, a W-score was calculated based on the normal distribution of an independent reference group of CN older adults (n = 77; age range = 45-89). In this reference group, hippocampal volume was regressed onto age, gender, and total ICV, yielding residuals that were converted to percentiles and subsequently standardized (i.e., converted to z-scores). The upper and lower tails of this normal distribution were used to identify values that represent the highest and lowest hippocampal volumes within the CN reference sample. From this, a W-score was derived that accurately predicted AD diagnosis with 85% sensitivity in a second independent sample of 51 individuals with AD (W-score: < -0.9063) (Snitz et al., in *press*). In other words, W-

scores can be considered age, gender, and ICV adjusted Z-scores, and therefore negative W-scores represent adjusted volumes below the expected mean for the CN reference sample (Jack et al., 2008). Thus, in the proposed study, neurodegeneration was included as a moderator and was represented using a binary variable that reflected subjects that do not reach the pre-identified threshold (ND-) and those that reach or exceed an ICV-adjusted W-score of -0.9063 (ND+).

2.3.2 Positron Emission Tomography

PET imaging data was collected following the MRI sequence. Using a Siemens/CTI ECAT HR+ scanner, PiB-PET data acquisition began 50 minutes after participants were injected with 15 +-1.5 mCi of PiB in 3-dimensional imaging mode (2.4 mm slice width, 63 planes)(Lopez et al., 2014; Snitz et al., 2013). PET data were reconstructed using filtered back-projection, with a final PET image resolution of ~6 mm (transverse and axial) (Lopez et al., 2014; Mathis et al., 2013; Snitz et al., 2013). PiB retention was scaled to the injected dose and body mass of each participant to yield standardized uptake values (SUV) (Snitz et al., 2013). An SUV ratio (SUVR) was calculated using PiB uptake in the grey matter tissue of the cerebellum as a reference region (Snitz et al., 2013). The cerebellum is a commonly used reference region when assessing amyloid binding, as Aβ plaque accumulation is typically negligible in the cerebellum.

Following PiB-PET data acquisition, PET images were co-registered to MR images to facilitate region of interest (ROI) segmentation. The SUVRs within 6 bilateral ROI's (anterior cingulate, anterior ventral striatum, frontal lobe, lateral temporal cortex, precuneus, and parietal cortex) were averaged to reflect a global estimate of PiB retention (mean SUVR) (Lopez et al., 2014). This continuous variable served as the primary dependent variable in statistical models investigating patterns of Aβ plaque deposition.

There are two methods of delineating the aforementioned ROI's, which include a manual approach and an automated approach using established software and template image. Both manual and automated approaches are commonly used and results of these approaches are highly correlated, however, at the time of data collection in 2011, manual segmentation was considered the most precise method (Rosario et al., 2011). Each approach was employed in the present study, with manual segmentation applied in the subjects with longitudinal data and automated segmentation used in the full baseline sample. Inter-rater reliability of manual tracing obtained across 3 raters demonstrates substantial reliability (intraclass correlation coefficient ≥ 0.932) (Rosario et al., 2011).

Automated approaches are often used for efficiency purposes, particularly in studies with large sample sizes, as manual segmentation is quite labor intensive (Rosario et al., 2011). In the present study, the baseline PET data (n = 194) collected in 2009 was processed using an automated segmentation approach. Specifically, ROI's were manually drawn on a high-resolution MR image of a female older adult (79 years) with MCI (Mathis et al., 2013; Snitz et al., 2013). This was intentionally chosen as the template image as it represented a moderate degree of brain atrophy (Jagust et al., 2010). Using Statistical Parametric Mapping (SPM8) software, each subjects PET and MRI data were co-registered, and the MR image was subsequently normalized to the MCI template (Rosario et al., 2011). The parameters used to normalize the high-resolution MR image to the MCI template were then applied to each subject's PET data, resulting in spatial normalization of the PET data to the MCI template (Rosario et al., 2011). The ROI's drawn on the template image were then applied to each subjects normalized PET image, generating SUVR estimates for each ROI (Jagust et al., 2010; Rosario et al., 2011). This yielded 10 distinct ROI's from which PiB retention in the grey matter tissue was estimated, including the anterior cingulate

gyrus, frontal cortex, precuneus, anterior ventral striatum, lateral temporal cortex, mesial temporal cortex, occipital cortex, occipital pole, sensory-motor cortex, and parietal lobe.

In the 103 subjects that underwent PET imaging at baseline *and* follow-up, a manual ROI delineation approach was employed. Using this method, each participants MR and PET data were co-registered, and each subject's native MR space was used to generate the ROIs. Thus, manual ROIs were hand drawn using various anatomical criteria by trained raters, based on each individual subjects structural MR images. Co-registration of the MR and PET images allowed these manually drawn ROI's to be applied to the PET data, from which continuous SUVR estimates in each ROI were obtained (Rosario et al., 2011). The manual approach resulted in 16 bilateral ROIs including the anterior cingulate gyrus, anterior ventral striatum, dorsal frontal cortex, frontal cortex, lateral temporal cortex, mesial temporal cortex, occipital cortex, occipital pole, pregenual anterior cingulate, parietal cortex, global precuneus and precuneual divisions (lower precuneus, middle precuneus, and upper precuneus), ventral frontal cortex, and sensory-motor cortex. A measure of global PiB retention reflects the average signal across the anterior cingulate, frontal cortex, lateral temporal cortex, parietal, precuneus and anterior striatum ROIs. In all models, continuous estimates of PiB retention were used as dependent variables in order to maximize power and explore global and regional associations that may not be captured using conventional threshold-based classification methods (Grothe et al., 2017; Jansen et al., 2018; Villeneuve et al., 2015).

2.4 MEASURES

2.4.1 Neuropsychological assessments

Participants in the GEMS Neuroimaging Sub-Study completed annual neuropsychological assessments, beginning in 2009. The neuropsychological evaluation included the Mini-Mental State Examination (MMSE), as well as measures of memory, (California Verbal Learning Test, Rey-Osterrieth Figure Test), language (semantic (animals) and phonemic fluency (FAS)), processing speed (Trail Making Test, Part A), and executive function (Trail Making Test, Part B) (Snitz et al., 2013). This battery was subsequently administered again at the two-year follow-up (in 2011), and at both baseline and follow-up, these assessments were completed within 6 weeks of neuroimaging acquisition. Cognitive adjudication was completed by the GEMS Cognitive Diagnostic Center, which took into consideration historical serial cognitive assessments obtained during the course of the GEMS trial, but were blinded to the baseline and follow-up neuroimaging results (Mathis et al., 2013; Snitz et al., 2013). Performance on 1 to 3 cognitive tests that exceeded 1.5 standard deviations below age- and education-adjusted means was required for a diagnosis of MCI (Lopez et al., 2014; Snitz et al., 2013). A diagnosis of dementia required impaired performance on measures in two or more cognitive domains, with deficits significant enough to interfere with an ability to independently complete activities of daily living (Lopez et al., 2014). Consensus between a neurologist, a psychiatrist, and a neuropsychologist was required for a cognitive diagnosis (Snitz et al., 2013). Statistical models of cognitive outcomes will either include continuous measures of memory performance or binary/categorical variables reflecting cognitive status, based on the diagnoses given at baseline or follow-up (CN vs. MCI).

The present study included two measures of memory performance: the California Verbal Learning Test (CVLT) and the Rey-Osterreith Complex Figure Test (Rey-O). Despite the availability of a full neuropsychological battery, we chose to focus on memory tasks in the present study 1) because delayed memory deficits are one of the earliest cognitive indicators of AD onset and 2) to prevent type 1 error inflation by reducing the number of analyses conducted. On the CVLT, participants were read aloud a 16-item list of words and asked to repeat the words from the list, with the total number of words recalled over 5 consecutive trials used as an estimate of list learning/immediate recall. After being read a distractor list, participants were asked to freely recall the original word list. After a 20-30 min delay, the participants were asked again to freely recall as many items from the 16-word list as possible. The total number of correct items (range 0 – 16) recalled was used as a continuous measure of delayed verbal memory. The cued recall trials were not included in the present analysis. Participants also completed the Rey-O, a measure of immediate and delayed visual memory. On this task, participants were asked to copy a complex figure comprising of a number of distinct elements. Participants were then asked to draw the figure from memory immediately after the copy trial, and once again 20-30 minutes later. A 36-point summary score, based on the accuracy of the 18 units/component contained within the figure was used to quantify immediate and delayed visuospatial memory performance. Each measure was converted to z-scores using standard deviations.

2.4.2 Inflammatory blood assays

Participants were asked to fast and to avoid exercise and alcohol for 12 hours prior to blood draw. Blood samples were collected at the GEMS closeout visit between October 2007 and March 2008 and stored at -70ºC until analyzed (Hughes, Lopez, et al., 2014). Soluble CD14, CRP, ad IL-6

were measured using an enzyme-linked immunosorbant assay (ELISA) kit according to the manufacturers instructions (R&D Systems). The soluble receptors for TNF-α (TNF-αRI and TNF-αRII) were measured using a Multiplex Panel (Millipore). Since TNF-α is cleared fairly quickly from circulation, soluble receptor levels reflect more stable measures of TNF-α activity.

These inflammatory biomarkers will be used to predict cognitive status and Aβ pathology approximately 10 months after measurement and again 3 years following blood draw. Numerous longitudinal studies have measured the reliability of inflammatory markers over the course of months and years, and have found the biomarkers included in the present study to be moderately-to-highly stable over time. Table 1 summarizes existing studies that have assessed the reliability of these particular biomarkers and includes information on cohort size, baseline age, follow-up time and estimate of stability over time, measured using either spearman's correlation coefficient (r) or intraclass correlation coefficients (ICC) for each study.

Table 1. Reliability of peripheral biomarkers included in the present study.

Biomarker	Study	Sample Size	Age at Baseline	Follow-up	ICC/Corr.
CD 14	Epstein, 2013	200	21 - 55 (median 35)	2 years	ICC = 0.57
	McKay, 2017	223	18.7-74.5 (median 45.6)	1.9 years	ICC = 0.52
	Catelier, 2008	112	71.7	4-8 wks apart	r = 0.60
sTNF-RI	Clendenen, 2010	18	42-62	2 years	ICC = 0.31
	Hardikar, 2014	360	53 - 69 (61)	1.8 years	ICC = 0.89
	Navarro, 2012	62	20-40	4-6 months	ICC = 0.92
	Gu, 2010	65	35-65 (mean 50.8)	2 years	ICC = 0.68
sTNF-RII	Clendenen, 2010	18	42-62	2 years	ICC = 0.68
	Epstein, 2013	200	21 - 55 (median 35)	2 years	ICC = 0.73
	Hardikar, 2014	360	53 - 69 (median 61)	1.8 years	ICC = 0.85
	Navarro, 2012	62	20-40	4-6 months	ICC = 0.90
	Gu, 2010	65	35-65	2 years	ICC = 0.80
	McKay, 2017	223	18.7-74.5 (median 45.6)	1.9 years	ICC = 0.71
IL-6	Alley, 2007	657	65-94 (mean 73.3)	3 years	no avg. change
	Clendenen, 2010	18	42-62	2 years	ICC = 0.81
	Epstein, 2013	200	21 - 55 (median 35)		ICC = 0.55
	Gu, 2010	65	35-65 (mean 50.8)	2 years	ICC = 0.92
	McKay, 2017	223	18.7-74.5 (median 45.6)	1.9 years	ICC = 0.60
	Metti, 2015	135	70-79	10 years	r = 0.47
	Nash, 2013	1,443	53-80+	10 years	% change = 14.8

Citations for studies listed above: (Alley, Crimmins, Bandeen-Roche, Guralnik, & Ferrucci, 2007; Catellier, Aleksic, Folsom, & Boerwinkle, 2008; Clendenen et al., 2010; Epstein et al., 2013; Gu et al., 2009; Hardikar et al., 2014; Jackson et al., 2015; McKay et al., 2017; Metti et al., 2015; Nash et al., 2013; Navarro et al., 2012; Out, Hall, Granger, Page, & Woods, 2012).

2.4.3 Measures of covariates of interest in the current study

2.4.3.1 APOE genotyping

APOE genotyping of 3 major allelic forms of APOE (ε2, ε3, ε4) was performed on isolated DNA from plasma samples collected at the GEMS closeout visit (Hughes, Lopez, et al., 2014; Mathis et al., 2013) Frozen plasma samples were thawed and analyzed using an immunoturbidimetric procedure developed by Kamiya Biomedical Company (Hughes, Lopez, et al., 2014). A binary variable was created in which non-carriers were assigned a value of 0 and heterozygous and homozygous APOE ε4 carriers received a value of 1.

2.4.3.2 Lipid assays

Stored plasma samples from the 2008 GEMS closeout visit were thawed and used to estimate triglyceride, total cholesterol, and high-density lipoprotein (HDL) cholesterol concentrations (Hughes, Lopez, et al., 2014). Conventional enzymatic methods were used to estimate lipid concentrations (Hughes, Lopez, et al., 2014), and low-density lipoprotein cholesterol levels were measured by the Friedewald equation (Friedewald, Levy, & Fredrickson, 1972). All apolipoprotein and lipid analyses were completed at the Heinz Nutrition Laboratory in the Department of Epidemiology at the University of Pittsburgh.

Assessment for a history of cardiovascular disease was conducted, and a binary measure indicating a history of cardiovascular disease was created, and included myocardial infarction, congestive heart failure, coronary revascularization procedures, angina pectoris and peripheral vascular disease. Variables were also calculated categorizing participants based on the presence of hypertension (0 = absent, 1 = present) and diabetes (0 = absent, 1 = present) at baseline.

2.4.3.3 Self-reported depressive symptoms

Participants completed the 10-question Center for Epidemiologic Studies Depression Scale (CES-D), yielding a continuous estimate of self-reported depressive symptoms, with higher values reflecting greater symptom burden.

2.5 DATA ANALYSIS

Ordinary least squares linear regression models were employed to investigate the cross-sectional and longitudinal associations between baseline inflammatory biomarkers, memory performance,

and Aβ plaque deposition/progression. As cognitive status was represented by a binary (CN or MCI), logistic regression models were used to assess the relationship between baseline inflammatory biomarkers and cognitive status at baseline and follow-up. Normality was examined for all variables, of which serum CD14, IL-6, and duration of time (weeks) between blood draw and PET imaging showed significant positive skew. These measures were normalized by logarithmic transformation prior to analysis. All models were adjusted for age, gender, and years of education. Outliers were addressed using the winsorizing approach. For each variable, values that exceeded 3 standard deviations above or below the mean were identified and manually modified, such that outliers were assigned values that corresponded to the 95^{th} or 5^{th} percentiles, respectively. This is a conventional approach to addressing outliers, which allows us to retain subjects within the outlier range and continue to reflect their values in the upper/lower tails of the distribution while also minimizing skew.

A strength of this study is the availability of multiple inflammatory biomarkers including pro-inflammatory cytokines (or their corresponding receptor concentrations) IL-6, TNF-αRI, TNF-αRII, and cell surface receptor CD14. Given that little is known about the particular inflammatory markers associated with Aβ pathology, separate but identical statistical models were run for each inflammatory biomarker. To reduce the number of models run, concentrations of TNF-αRI and TNF-αRII were summed and averaged to reflect an overall estimate of soluble TNF-α receptor concentrations. Although a small number of rodent models suggest that these two TNF-α receptors may have different mechanisms of action in the CNS (Ramesh et al., 2013), the existing data is limited and this difference may not be reflected in peripheral receptor concentrations. Thus, each inflammatory variable served as continuous predictors in independent statistical models. Table 2 summarizes 1) the mechanisms of action of each of these inflammatory biomarkers under

conditions of immune challenge, 2) the evidence from animal and human studies that implicate these biomarkers in Aβ pathogenesis or AD onset, 3) the hypothesized relationship between each biomarker and the outcome variables of interest in the proposed study, and 4) the rationale used to support each hypothesis.

Another strength of this study is the availability of both cognitively healthy participants and those with MCI, and the ability to discriminate between these subgroups. Widespread amyloid deposition and neurodegenerative changes take place prior to the onset of clinically detectable cognitive deficits, and therefore the preclinical phase, as defined by the NIA-AA, includes those exhibiting AD-related pathology but that do not meet diagnostic criteria for MCI. In order to evaluate these relationships at the earliest possible point, it is critical to focus on populations that have not yet progressed to MCI. Therefore, all of the models described below were conducted in the combined sample (MCI + CN), and repeated after excluding subjects with MCI. In other words, all cross-sectional and longitudinal analyses conducted in the full samples were replicated in the subsample of CN participants, which allowed us to determine whether hypothesized relationships were observed in, or were specific to, asymptomatic populations. In using this approach, we aimed to gain a better understanding of the temporal relationship between inflammatory biomarkers and amyloid burden.

Table 2. Inflammatory biomarkers, existing research, and specific hypotheses in the proposed study.

	Properties and functions	**Extant Research**	**Hypothesis**	**Rationale**
CD14	Cell-surface receptor expressed by microglia; LPS receptor, monocyte activator	**Animals:** CD14 facilitates binding of microglia to Aβ oligomers and fibrils, resulting in microglial activation (Heneka, 2015) and downstream inflammatory processes; knockdown of cell-surface receptors reduces cytokine production and Aβ aggregation In the PNS, binding of CD14 to LPS triggers synthesis and production of IL1β, TNF, IL-6 (Zamani et al., 2013)	Elevated levels of CD14 will be positively associated with MCI status at baseline, conversion to MCI/dementia at follow-up, and greater Aβ deposition at baseline and follow-up	Facilitates activation of monocytes in PNS, may broadly reflect greater inflammatory burden with Aβ pathology; given the initial role of CNS CD14 in promoting the binding of Aβ to microglia, may also be up-regulated in periphery
TNF-α (RI & II)	Pro-inflammatory cytokine released by activated microglia, macrophages, neurons; mediates release of neurotoxic substances (ROS, iNOS), apoptosis, activates astrocytes	**Animals:** TNF-α overexpressed in AD transgenic mice (Wang, 2015); Aβ species promote production of TNF-α (Heneka, 2015); blocking TNF-α signaling reduces Aβ plaques (Wang, 2015); TNF-α contributes to APP processing (Wang 2015), stimulates release of reactive nitrogen species (Rubio-Perez, 2012), impairs BBB integrity (Calsolaro, 2016), and penetrate BBB from periphery **Humans:** elevated levels of TNF-α surrounding amyloid plaques in brain tissue of AD patients (Calsolaro, 2014); elevated peripheral levels in individuals with AD (Swardfager 2010), greater serum TNF-α levels assozciated with more rapid rate of decline over 6 months (Walters, 2015) Peripheral concentrations elevated in metabolic syndrome (Srikanthan, 2014)	Elevated levels of TNF-α (RI and RII) be positively associated with MCI status at baseline, conversion to MCI/dementia at follow-up, and greater Aβ deposition at baseline and follow-up Will positively mediate relationship between CVD risk factors and Aβ deposition	In rodents, TNF-α perpetuates Aβ, impairs BBB; in humans elevated in post-mortem brain tissue of AD, increased peripheral concentrations assoc. with cognitive impairment Mediating effect due to TNF-α associations with obesity, insulin resistance, metabolic syndrome
IL-6	Pro-inflammatory cytokine produced by microglia and astrocytes in CNS; macrophages, T cells, adipose tissue in PNS; induces acute-phase response; in PNS may inhibit TNF-α production; in CNS activates microglia, promotes astrogliosis, release of cytotoxic substances	**Animals:** IL-6 activates microglia/macrophages; Aβ species promote production of IL-6 (Heneka, 2015); IL-6 contributes to APP processing (Wang, 2015), stimulates release of reactive nitrogen species (Rubio-Perez, 2012), able to impair BBB integrity (Calsolaro, 2016) and penetrate BBB from the periphery **Humans:** elevated levels of IL-6 surrounding amyloid plaques in brain tissue of AD patients (Calsolaro, 2014); elevated peripheral concentrations in individuals with AD(Swardfager et al.,); predicts risk of incident dementia 3-8 years later (Engelhart, 2004) Peripheral concentrations elevated in metabolic syndrome (Srikanthan, 2014)	IL-6 will be positively associated with MCI status at baseline, conversion to MCI/dementia at follow-up, and greater Aβ deposition at baseline and follow-up Will positively mediate relationship between CVD risk factors and Aβ deposition	In animals, IL-6 contributes to APP processing, microglial priming, impairs BBB, can penetrate BBB from periphery; in humans elevated in post-mortem brain tissue of AD, predicts future risk of incident dementia, suggesting possible role of IL-6 early in disease course Mediating effect due to associations with obesity, insulin resistance, metabolic syndrome

Aim 1 assessed the cross-sectional associations between baseline inflammatory markers, cognitive status, and memory performance (n = 173). Using logistic regression models, cognitive status was represented by a binary variable reflecting CN subjects and those with a baseline diagnosis of MCI, using CN participants as the reference group. A series of linear regression models assessed the relationship between each inflammatory marker and memory performance. To this end, each subjects' performance on a visual memory task (Rey-O) and a verbal memory task (CVLT) was z-scored, summed, and averaged to yield a continuous composite measure of global or general memory performance. To distinguish associations with verbal versus visual memory performance, two additional z-scores were calculated, one which reflects average performance on immediate and delayed trials of the CVLT, and the second representing performance on the Rey-O.

Aim 2 assessed the cross-sectional associations between baseline inflammatory markers and fibrillar Aβ plaque deposition (n = 173). This model also examined whether the relationship between inflammatory markers and Aβ plaque deposition varied as a function of preclinical disease stage. According to the NIA-AA guidelines, an Aβ+/ND- imaging profile represents the initial preclinical stage, while the combination of significant Aβ deposition and neurodegeneration reflects the second neuropathological phase of preclinical AD. Hippocampal atrophy was used as a proxy for neurodegeneration, and was included as a moderator in linear regression models. In other words, consistent with other cross-sectional studies (Bilgel et al., 2017, Mormino et al., 2014, Zhao et al., 2018), hippocampal atrophy served as an indicator of where subjects may fall on the preclinical continuum. Thus, along with covariates, each model included a continuous inflammatory marker, a binary variable (W-score) representing neurodegeneration status (ND+ vs. ND-), and their interaction product. Along with W-score, hippocampal volume, calculated as a

proportion of total ICV (e.g., sum of right and left hippocampi/ICV), was included in separate moderation models to provide a continuous estimate of hippocampal atrophy. Thus, regression models included age, gender, and education level as covariates, inflammatory biomarker and hippocampal atrophy as independent predictors, and their interaction product (inflammatory biomarker X hippocampal atrophy). A continuous measure of global amyloid burden was used as the primary dependent variable in main effect and moderation models.

Given that, to our knowledge, only one existing study has examined associations between peripheral inflammatory biomarkers and regional PiB uptake in humans, we also assessed whether these relationships were global or spatially distinct. Therefore, for inflammatory variables that demonstrated significant main or interaction effects on *global* PiB-PET retention, subsequent secondary hierarchical regression analyses were run using template-based ROIs to assess the possible regional specificity of these associations. Given 1) that this is a preclinical/MCI sample and 2) the established hierarchical nature of early amyloid progression, we hypothesized that the relationship between peripheral biomarkers and PiB uptake would be specific to (or strongest in) regions particularly vulnerable to Aβ accumulation in the earliest phases of amyloidosis, which included 3 ROI's: the anterior cingulate gyrus, frontal cortex, and precuneus. These were considered *a priori* ROIs, and thus the alpha threshold for significance was set at 0.05. Owing to the exploratory nature of this work, we also assessed whether these relationships were apparent in other cortical and subcortical regions. This included 8 additional template-derived ROIs (anterior ventral striatum, lateral temporal, mesial temporal cortex, occipital cortex, occipital pole, parietal cortex, sensory-motor cortex, and the thalamus). To prevent type 1 error inflation, a Bonferonni threshold was applied to models including the 8 ROI's considered exploratory or *post hoc*, resulting in a corrected p-value of 0.004 (e.g., 0.05/8).

Finally, to examine the influence of potential confounding variables including comorbid cardiovascular and health factors, each model was re-run adjusting for demographic characteristics as well as history of heart disease, diabetes, and hypertension, all represented by binary variables reflecting the presence or absence of each health factor, and a continuous measure of self-reported depressive symptoms. Given the association between peripheral inflammatory biomarkers and white matter lesions, white matter hyperintensities (a continuous measure quantified as volume of white matter lesions/total brain volume) were controlled for in secondary analyses. Also entered was a variable reflecting time since blood draw, which was calculated as the number of weeks between blood sample collection (from which inflammatory biomarkers were quantified) and baseline PiB-PET neuroimaging. Given the variability of inflammatory biomarker expression over time, this allowed us to adjust for individual differences in the time that had elapsed between blood sample collection and image acquisition. Finally, there is a well-established and robust association between APOE ε4 carrier status and Aβ burden (Kantarci et al., 2012; Lim, Ellis, et al., 2013), and we explored whether the relationship between inflammatory factors and PiB retention existed independently of APOE ε4 genotype. Therefore, participants were dichotomized into non-carriers and carriers (combining heterozygous and homozygous carriers) and included in a third set of sensitivity models to explore whether observed associations remained after additional adjustment for APOE ε4 status.

Aim 3 leveraged longitudinal data collected in a subsample of participants to investigate whether inflammatory biomarkers predicted conversion of cognitive status or change in memory performance over the two-year follow-up period (n = 90). A series of logistic regression models were used to assess whether each inflammatory biomarker predicted cognitive diagnosis at follow-

up (e.g., MCI or CN at follow-up in a combined longitudinal sample), as well as conversion from CN to MCI among those that were cognitively healthy at baseline.

Residualized change scores were calculated and used as dependent variables in linear regression models, to explore the relationship between inflammatory markers and longitudinal change in memory performance from baseline (T_1) to follow-up (T_2). Thus, for each outcome variable of interest, cognitive performance at T_2 was regressed on T_1 to generate a residual. The estimated residuals reflected the deviation between the predicted value (based on T_1) and the actual value (T_2), thus reflecting change from T_1 to T_2. This approach is preferable to a raw difference score as residualized change scores estimate change over time while also adjusting for the values of each outcome variable of interest at baseline. Estimating change over time after correcting for baseline levels of each variable provides a purer measure of change that is unaffected by individual differences in variables of interest at baseline.

Aim 4 examined whether baseline serum concentrations of inflammatory mediators predicted progression of Aβ plaque deposition over the 2-year follow-up (n = 90). To this end, change in Aβ deposition between the two consecutive scans was estimated by regressing PiB retention at T_2 on T_1. This resulted in unstandardized residuals for global and regional PiB uptake, which were used as dependent variables in longitudinal models and reflect change in Aβ plaque deposition after adjusting for individual differences in Aβ pathology at baseline. Baseline hippocampal atrophy was included as a moderator in statistical models, as well as the hippocampal volume X inflammatory biomarker interaction term, to assess whether the relationship between each biomarker and Aβ progression was greater in magnitude among subjects that also exhibited significant hippocampal atrophy. The regional specificity of these relationships was also investigated in longitudinal models. A hand-drawn region of interest approach was applied to the

PiB-PET data of subjects that completed both the baseline and follow-up scans, which yielded more distinct ROI's than the template-based ROI approached used in the full baseline sample (e.g., anterior cingulate ROI, superior anterior cingulate ROI, and pregenual anterior cingulate ROI). Therefore, a two-tailed alpha of $p = 0.05$ was used to define statistical significance for those ROI's considered *a priori* (e.g., precuneus and frontal ROIs), while a Bonferroni correction threshold was applied to any remaining ROI's ($0.05/7 = 0.007$) assessed. To further reduce the number of models, only ROI's that exhibited main or interaction effects in cross-sectional analyses were evaluated in longitudinal models. Among models that achieved significance when controlling for participant demographics, follow-up hierarchical analyses were conducted that further adjusted for Aβ-relevant factors, including cardiovascular risk factors, mood, white matter lesions, and APOE genotype.

3.0 RESULTS

3.1 CROSS-SECTIONAL RESULTS

3.1.1 Subject characteristics

Of the 194 participants included in the GEM Neuroimaging Substudy, 190 had complete blood data collected in 2008, from which serum concentrations of inflammatory biomarkers were quantified. Four subjects were missing inflammatory data and were excluded from analyses. Another 5 participants had C-reactive protein values that exceeded 10 mg/L, suggesting that they may have been ill at the time of blood draw, and were therefore excluded from analyses. An additional 8 subjects were missing intracranial volume data and were excluded, as this was required to calculate the proportion of hippocampal volume to total brain volume. Finally, in order to focus exclusively on subjects without dementia, the 4 participants that met criteria for dementia during the baseline 2009 neuropsychological assessment were excluded from all analyses. The final baseline sample consisted of 173 participants.

The average age of the 173 participants included in the present study was 85.45 (SD = 2.84), with ages ranging from 82-95. Women comprised 40.5% of the sample (N = 70), all but 6 subjects were Caucasian (96.5%), and participants had an average of 14.64 years of education (SD = 2.65). Thirty-four participants were diagnosed with MCI at baseline (19.7%), including 24 with amnestic MCI and 10 with non-amnestic MCI. Ninety-four participants (54.3%) were classified as amyloid positive and 79 (45.7%) were considered amyloid negative using a standard iterative outlier method (defined as PiB+ if global Aβ > 1.57) (Nadkarni et al., 2017). Of the full sample,

33 subjects (19.1%) were carriers of the APOE ε4 genotype. Associations between predictors and covariates of interest were assessed using two-tailed t-tests and bivariate correlations. IL-6 was moderately correlated with CD14 ($r = 0.30$; $p < 0.001$) and TNF-α ($r = 0.27$; $p < .001$). The relationship between CD14 and TNF-α was moderate but slightly larger in magnitude ($r = 0.37$; $p < 0.001$) (Table 3). Baseline characteristics, including, age, education level, gender, APOE carrier status, history of heart disease, and presence of diabetes or hypertension, did not differ between the CN subjects (N = 139) and those with MCI (N = 34). As would be expected, cognitively healthy participants performed significantly better on the MMSE ($t = -4.21$; $p < 0.001$) and had larger hippocampal volumes on average ($t = -4.08$; $p < 0.001$) relative to those with MCI. Table 4 includes the descriptive statistics for the combined baseline sample and the CN and MCI subsamples. Even prior to adjusting for demographic factors, there was no significant relationship between hippocampal volume and any of the 3 inflammatory biomarkers in bivariate correlation models. This is the case for the full sample, and the CN subsample.

Table 3. Bivariate correlations between inflammatory biomarkers in the full baseline sample (N = 173).

	IL-6	CD14	TNF-α (avg)	TNF-α R1	TNF-α RII
IL-6	1				
CD14	.299*	1			
TNF-α (avg)	.271*	.370*	1		
TNF-α R1	.231*	.255*	.782*	1	
TNF-α RII	.264*	.374*	.990**	.688**	1

*Correlations are significant at 0.01 (2-tailed).

Table 4. Demographic, inflammatory, health, and cognitive characteristics for combined baseline sample and the CN and MCI subsamples.

	Combined Sample N = 173	**CN** N = 139	**MCI** N = 34
	% (N) or *M (SD)*	% (N) or *M (SD)*	% (N) or *M (SD)*
Age	85.45 (2.84)	85.36 (2.84)	85.79 (2.83)
Gender (% F)	40.5 (70)	43.2 (60)	29.4 (10)
Education	14.64 (2.65)	14.79 (2.65)	14 (2.58)
ApoE, % (N)	19.1 (33)	19.4 (27)	17.6 (6)
Hx Heart Disease, % (N)	17.3 (30)	15.1 (21)	26.5 (9)
Diabetes, % (N)	5.8 (10)	5.2 (7)	8.8 (3)
Hypertension, % (N)	34.1 (59)	32.4 (45)	41.2 (14)
MCI, % (N)	19.7 (34)	-	-
MMSE	27.62 (2.10)	28.04 (1.67)	25.94 (2.74)
CD14	1359.28 (261.04)	1367.61 (256.47)	1325.23 (280.37)
TNF-α	3842.16 (1077.41)	3791.89 (1013.95)	3980.75 (1147.61)
IL-6	2.63 (1.62)	2.56 (1.54)	2.9 (1.93)
Weeks from blood draw to PET	87.26 (38.34) range 50.4-331.1	88.84 (42.12) range 50.6 - 331	81.68 (13.95) range 58.4 - 115.6
PiB+ (%)	54.3 (94)	51.8 (72)	64.7 (22)
CVLT Immediate	43.10 (12.09)	45.7 (11.14)	32.47 (9.91)
CVLT Delay	8.25 (3.73)	9.19 (3.21)	4.38 (3.17)
Rey Immediate	16.18 (3.92)	17.01 (3.50)	12.77 (3.73)

3.1.2 Inflammatory biomarkers, cognitive diagnosis, and memory performance

3.1.2.1 Combined sample

Logistic regression models were employed to assess the association between inflammatory biomarkers and cognitive diagnosis at baseline. After adjusting for age, gender, and years of education, using CN subjects as the reference group, there were no significant associations between IL-6 (odds ratio (OR) = 1.17; p = 0.44), TNF-α (OR = 1.05; p = 0.81), or soluble CD14 (OR = 0.84; p = 0.40) and diagnostic status at baseline. These non-significant findings were unchanged after excluding the 10 subjects diagnosed with non-amnestic MCI.

Specific associations within the memory domain were also evaluated, using three continuous composite scores reflecting average performance on two memory tasks: the Rey-O and

the CVLT. This included an estimate of inter-task or global memory performance (average of immediate and delayed trials on both tasks), verbal memory performance (average of immediate and delayed CVLT trials), and visual memory performance (average of immediate and delayed Rey-O trials). Multivariable linear regression models revealed that elevated levels of IL-6 predicted poorer global memory performance ($p = 0.044$; $\beta = -0.148$; R^2 change = 0.021). Probing this relationship using task-specific composite scores revealed no significant association between IL-6 and Rey-O performance, and a trending relationship with performance on immediate and delayed trials of the CVLT ($p = 0.055$; $\beta = -0.133$; R^2 change = 0.017). Regression models including TNF-α and CD14 as independent predictors demonstrated no significant associations between each biomarker and cognitive performance in the combined sample. Hippocampal volume was positively associated with better performance on all z-score estimates.

3.1.2.2 Cognitively healthy subsample

These analyses were repeated in the subsample of subjects deemed CN at the time of imaging to assess associations between inflammatory biomarkers and memory performance exclusively among subjects without clinically significant cognitive impairment (e.g., those in the asymptomatic, preclinical phase). Among the 139 CN participants, the presence of elevated IL-6 was associated with worse global memory performance in adjusted models ($\beta = -0.187$; $p = 0.024$). In contrast to the findings in the combined sample, the inverse association between IL-6 concentrations and CVLT performance reached significance ($\beta = -0.171$; $p = 0.027$). The association between IL-6 and Rey-O performance remained non-significant ($\beta = -0.121$; $p = 0.161$).

Contrary to the null results observed in the combined sample, restricting the analysis to the 139 CN subjects revealed that lower peripheral concentrations of TNF-α were associated with

better verbal memory performance on the CVLT (β = -0.20; p = 0.015). There were no significant associations between TNF-α and the remaining memory composites (p > 0.05). CD14 was not associated with the global memory composite, nor did it predict task-specific performance. Parameter estimates from regression models predicting cognitive performance are included in Tables 5 and 6.

Table 5. Associations between IL-6 and cognitive performance on two memory tasks, after adjusting for age, gender, and years of education.

	Global Memory Performance	**Verbal Memory Performance (CVLT)**	**Visual Memory Performance (Rey-O)**
Combined Sample (MCI and Cognitive Healthy) N = 173			
IL-6	**P = 0.044;** **β = -0.148** **R^2 change = 0.021**	P = 0.055; β = -0.133 R^2 change = 0.017	P = 0.142; β = -0.113 R^2 change = 0.012
Cognitively Healthy Subjects (MCI subjects excluded) N = 139			
IL-6	**P = 0.024;** **β = -0.187** **R^2 change = 0.034**	**P = 0.027;** **B = -0.171** **R^2 change = 0.028**	P = 0.161; β = -0.121 R^2 change = 0.012

Bolded values are significant at p < 0.05.

Table 6. Associations between TNF-α and cognitive performance on two memory tasks, after adjusting for age, gender, and years of education. There were no significant TNF x hippocampal volume interactions.

	Global Memory Performance	**Verbal Memory Performance (CVLT)**	**Visual Memory Performance (Rey-O)**
Combined Sample (MCI and Cognitive Healthy) N = 173			
TNF-*α*	P = 0.148; β = -.114 R^2 change = 0.011	P = 0.197 β = -.096 R^2 change = 0.008	P = 0.256; β = -0.093 R^2 change = 0.007
Cognitively Healthy Subjects (MCI subjects excluded) N = 139			
TNF-*α*	P = 0.076; β = -0.157 R^2 change = 0.021	**P = 0.015;** **B = -0.20** **R^2 change = 0.034**	P = 0.628; β = -0.045 R^2 change = 0.002

Bolded values are significant at p < 0.05.

3.1.3 Main effects of inflammatory biomarkers on global and regional PiB retention

3.1.3.1 Combined sample

To explore the cross-sectional relationships between inflammatory mediators, neurodegeneration, and Aβ accumulation, hierarchical regression models were conducted including inflammatory biomarkers and hippocampal volume as independent predictors, as well as their interaction product. Identical regression models were conducted separately for each of the 3 inflammatory biomarkers. The main effects are summarized here, and the interaction effects are reported in the subsequent section. After adjusting for age, gender, and education level, hierarchical linear regression models revealed a direct association between soluble CD14 and global PiB uptake, such that higher serum concentrations of CD14 predicted greater global Aβ deposition ($\beta = 0.161$; $p = 0.039$). Holding hippocampal volume constant, there were no main effects of TNF-α ($p = 0.24$; $\beta = 0.98$) or IL-6 ($p = 0.226$; $\beta = -0.094$) on global PiB uptake. Hippocampal volume was not independently associated with global PiB SUVR retention.

The observed association between CD14 and global Aβ deposition warranted secondary analyses using template-derived ROI's to evaluate the possible regional specificity of these relationships. Therefore, multiple linear regression models were fit to explore associations between CD14 and continuous measures of regional PiB SUVR while controlling for age, gender, and education level. The three regions that were considered *a priori* ROI's, the anterior cingulate gyrus, frontal cortex, and precuneus, were assigned a standard significance threshold of $p < 0.05$. To adjust for multiple comparisons, a Bonferroni correction was applied to the remaining 8 ROI's, yielding an adjusted alpha set at $p < 0.004$. Secondary analyses showed that higher serum CD14 was positively associated with regional amyloid burden in the anterior cingulate gyrus ($\beta = 0.170$; $p = 0.029$), frontal cortex ($\beta = 0.173$; $p = 0.026$), and mesial temporal cortex ($\beta = 0.24$; $p = 0.002$)

(Figure 3). While positive associations between serum CD14 and Aβ deposition in the thalamus (β = 0.18; p = 0.020), occipital cortex (β = 0.18; p = 0.021), and occipital pole (β = 0.171; p = 0.030) were also observed, they did not survive correction for multiple comparisons.

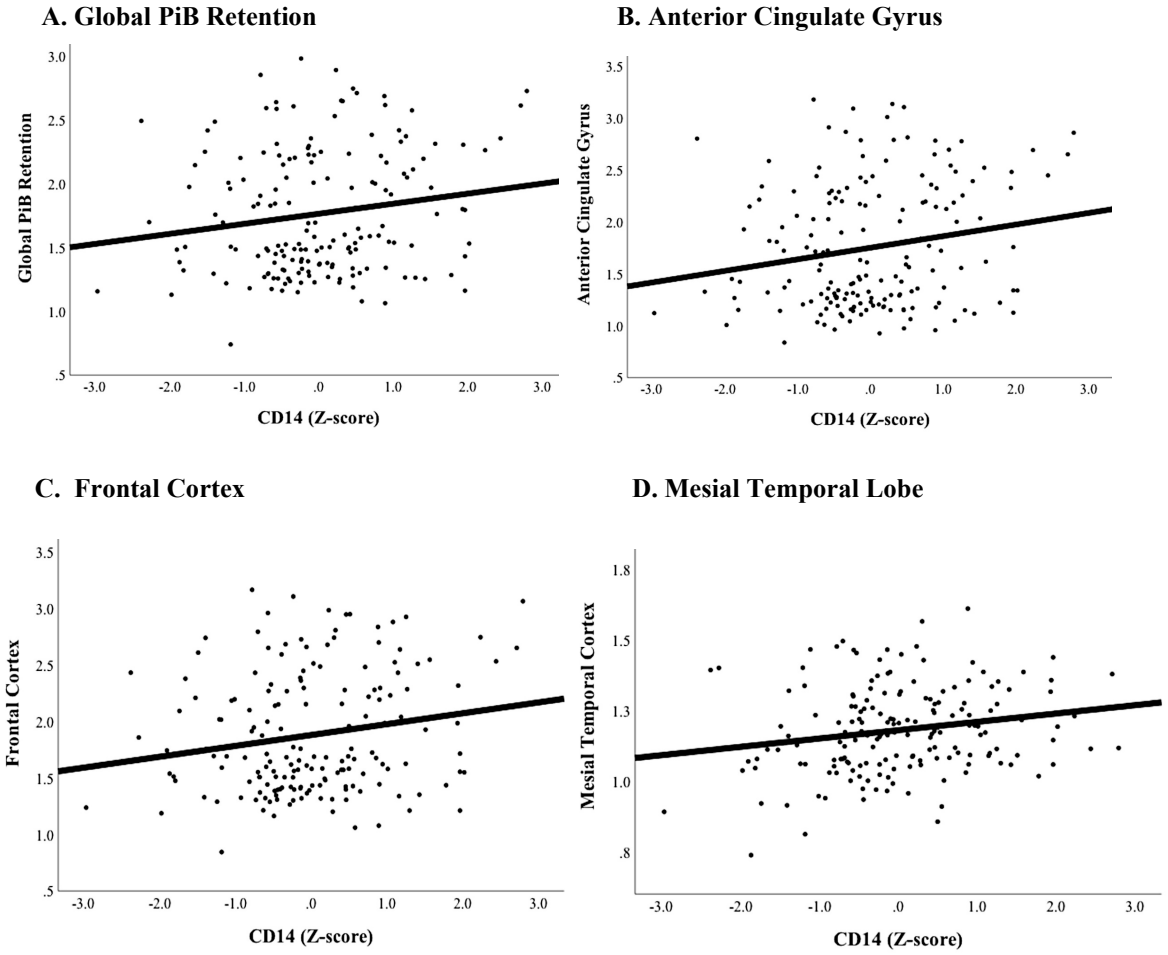

Figure 3. Cross-sectional associations between CD14 and global and regional PiB retention in the CN subsample (N = 139).

A series of exploratory models also evaluated whether the relationship between inflammatory biomarkers and PiB uptake existed independently of other factors known to predict amyloid burden, including vascular health, mood, and APOE ε4 genotype. Therefore, hierarchical

models controlled for demographic factors, history of heart disease, hypertension, diabetes, white matter hyperintensities, APOE genotype, depressive symptoms, and duration (weeks) between blood draw and PET imaging. Conservatively adjusted models included 145 observations, as some participants were missing APOE and/or white matter imaging data. The associations between soluble CD14 and global and regional PiB retention were no longer significant after controlling for the aforementioned covariates (all $p > 0.05$), even prior to adjusting for APOE ε4.

3.1.3.2 Cognitively healthy subsample

After excluding the 34 individuals with MCI, we found a trending, but non-significant association between CD14 and global PiB retention such that higher levels of CD14 were associated with greater global PiB retention ($\beta = 0.159$ $p = 0.068$). Regarding regional amyloid burden, the relationship between CD14 and PiB uptake in the anterior cingulate ($\beta = 0.175$; $p = 0.044$) persisted in the CN subsample, while this relationship no longer reached statistical significant in the frontal cortex ($\beta = 0.164$; $p = 0.059$). Elevated levels of CD14 were associated with greater amyloid burden in the mesial temporal lobe, although this relationship did not survive correction for multiple comparisons ($\beta = 0.230$; $p = 0.007$). None of the remaining ROIs were related to serum CD14, even prior to applying the Bonferroni correction. Consistent with the findings in the full sample, the total amount of Aβ deposition in the brain was not associated with either TNF-α or IL-6 in main effects models.

3.1.4 Inflammatory biomarkers, hippocampal volume, and global/regional PiB

3.1.4.1 Combined sample

To examine whether the association between inflammatory biomarkers and amyloid burden varied as a function of neurodegeneration, two estimates of hippocampal atrophy were included as moderators in hierarchical linear models. The first series of models incorporated W-score as a binary indicator of hippocampal neurodegeneration. Seven subjects were missing W-scores, therefore moderation models including this variable contained 166 observations. There were no significant interaction effects on global PiB-PET deposition for CD14, TNF-α, or IL-6 when binary W-score was used as a moderator (all $p > 0.05$). Given the lack of significant associations with global Aβ deposition, secondary moderation models evaluating regional PiB uptake were not conducted.

A second series of linear regression models were conducted using a continuous estimate of normalized hippocampal volume as a predictor and moderator in interaction terms. The relationship between TNF-α and global PiB retention was moderated by hippocampal volume, such that higher levels of TNF-α predicted elevated global amyloid burden among those with greater hippocampal atrophy ($\beta = -.172$; $p = 0.024$; R^2 change = 0.029). A significant interaction effect was also observed between IL-6 and hippocampal volume on global amyloid deposition ($p = 0.012$; $\beta = -.192$; R^2 change = 0.036). Hippocampal volume did not moderate the association between serum CD14 and global amyloid deposition ($\beta = -.104$; $p = 0.175$).

Significant interaction effects on global PiB retention merited secondary analyses exploring the conditional associations between inflammatory biomarkers and regional amyloid deposition as a function of hippocampal volume. Using template-derived ROI's, these analyses revealed a synergistic relationship between TNF-α and hippocampal volume on PiB retention in

the anterior cingulate ($p = 0.027$; $\beta = -.169$; R^2 change = 0.028), frontal cortex ($p = 0.033$; $\beta = -.163$; R^2 change = 0.026), and precuneus ($p = 0.027$; $\beta = -.168$; R^2 change = .028) ROIs. Interaction effects in the occipital cortex ($p = 0.024$; $\beta = -.170$; R^2 change = 0.028) and occipital pole ROI's ($p = 0.012$; $\beta = -.193$; R^2 change = 0.036) did not meet multiple comparison thresholds. Similar interaction effects were observed in models using IL-6 as a predictor, including associations with regional Aβ deposition in the anterior cingulate gyrus ($p = 0.015$; $\beta = -.184$; R^2 change = 0.033), frontal cortex ($p = 0.020$; $\beta = -.177$; R^2 change = 0.031), and precuneus ($p = 0.009$; $\beta = -.196$; R^2 change = 0.038). Associations were also observed in the occipital cortex ($p = 0.019$; $\beta = -.178$; R^2 change = 0.031) and the sensory-motor cortex ($p = 0.013$; $\beta = -.188$; R^2 change = 0.035), but were no longer significant after multiple comparison correction. Across all models, interaction terms explained an additional 2.5-5% of the variation in PiB retention, above and beyond covariates and independent predictors.

To probe significant inflammatory biomarker X hippocampal volume interactions, we chose several conditional values of hippocampal volume at which to evaluate the significance of the simple slopes for the regression of global PiB retention on IL-6 and TNF-α. To this end, the linear regression analyses were recomputed using hippocampal variables re-centered to reflect the degree of hippocampal atrophy. Thus, using separate statistical models, we assessed main effects of both IL-6 and TNF-α on Aβ deposition at the mean, 1 standard deviation above, and 1 standard deviation below the mean hippocampal volume. This analysis allowed us to examine where along the hippocampal volume continuum the slopes start to differ between those with varying levels of inflammatory biomarker expression. There was not a significant main effect of IL-6 or TNF-α in the regression models using mean-centered hippocampal volume ($p > 0.05$). In contrast, simple slope analyses revealed that, for both biomarkers, elevated concentrations of inflammatory

mediators predicted greater PiB deposition when hippocampal volume was mean-centered at 1 standard deviation below the mean (TNF-α: $p = 0.016$; $\beta = 1.22$; IL-6: $p = 0.008$; $\beta = 1.53$). There was also a main effect of both biomarkers when hippocampal volume was centered at 1 SD above the mean, such that global PiB retention remained stable across varying levels of cytokine expression among those exhibiting minimal hippocampal atrophy (TNF-α: $p = 0.017$; $\beta = 1.53$; IL-6: $p = 0.008$; $\beta = 1.94$). Thus, the relationship between inflammatory mediators and Aβ load varied as a function of hippocampal volume, such that elevated levels of both inflammatory markers (IL-6 and TNF-α) predicted greater Aβ deposition specifically among those that also displayed greater hippocampal atrophy (results from simple slope analyses depicted in Figures 4 & 5).

Notably, for both IL-6 and TNF-α, moderation models remained significant following additional adjustment for history of heart disease, hypertension, diabetes, white matter hyperintensities, depressive symptoms, and time from blood biomarker collection to PET imaging (N=156 for conservatively adjusted models due to reduced number of participants that completed white matter imaging). Interaction terms also withstood additional adjustment for APOE ε4 status in a third set of sensitivity models (N = 146). In hierarchical models, APOE ε4 genotype was the strongest predictor of global PiB retention, explaining 14.1% of the variance, followed by depressive symptoms (5.1% variance explained), and then interaction terms (IL-6: 3.6%; TNF-α: 2.9%). Thus, the amount of variance attributable to the interaction terms was greater than the combined variance explained by demographic characteristics (1%) and history/presence of cardiovascular risk factors (1.9% combined (hx heart disease, diabetes hypertension)).

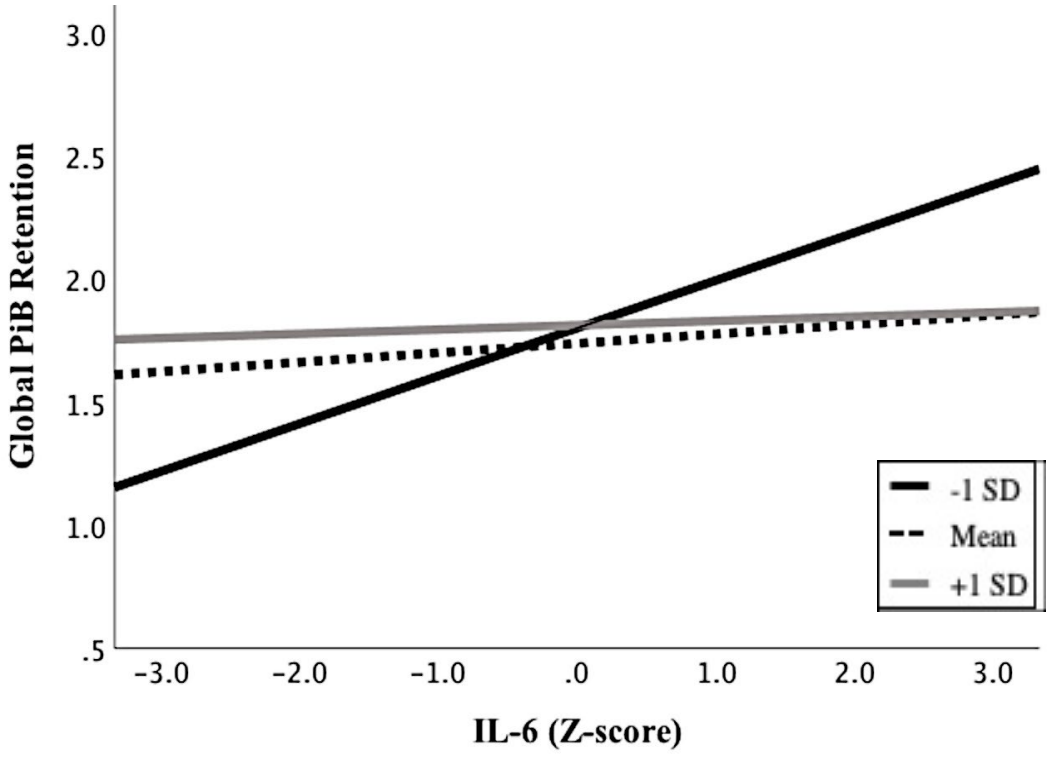

Figure 4. Results from simple slope analysis in the combined sample (N = 173), illustrating the linear associations between IL-6 and global PiB retention across several condittional values of hippocampal volume.

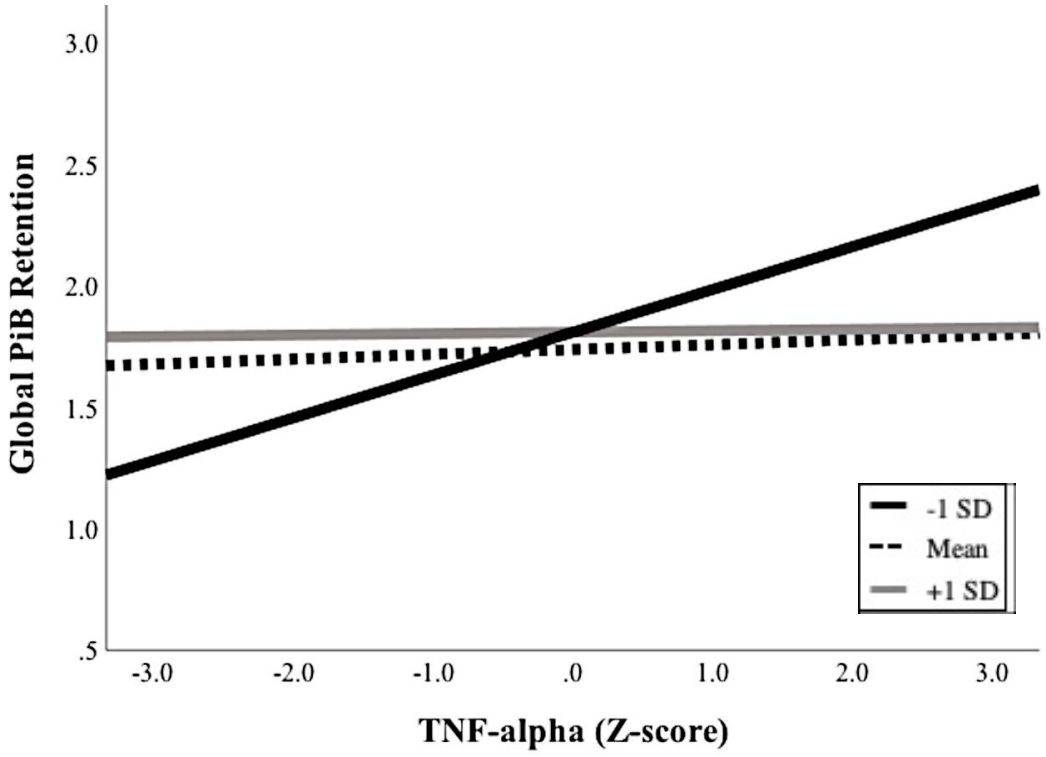

Figure 5. Results from simple slope analysis in the combined sample, illustrating the linear associations between TNF-α and global PiB retention across several conditional values of hippocampal volume.

Finally, we conducted a *post hoc* analysis to evaluate whether interaction effects would persist after restricting the analyses to the 94 subjects classified as PiB+ based on conventional dichotomous thresholds. Similar to the full sample, there was no main effect of hippocampal volume (β = -0.146; p = 0.198), IL-6 (β = 0.142; p = 0.21) or TNF-α on global PiB uptake (β = -.03; p = 0.783). The interaction between IL-6 and hippocampal volume remained significant (β = -.224; p = 0.033). Despite the reduced sample size, the amount of variance explained by the interaction term was larger than that observed in the full sample (R^2 = .048). The TNF-α interaction term failed to reach statistical significance (β = -.097; p = 0.361; R^2 change = .009).

3.1.4.2 Cognitively healthy subsample

The interaction between TNF-α and hippocampal volume on global PiB retention remained significant when restricting the analysis to cognitively healthy participants ($p = 0.027$; $\beta = -0.191$; R^2 change = .036). Interaction terms were also significant with respect to regional PiB uptake in the anterior cingulate cortex ($p = 0.040$; $\beta = -.178$; R^2 change = .031), frontal cortex ($p = 0.039$; $\beta = -.178$; R^2 change = .031), and precuneus ($p = 0.010$; $\beta = -.220$; R^2 change = .048). The interaction terms were not significant for the remaining regional PiB-PET ROIs. Parameter estimates and statistical significance remained stable in all models after additional adjustment for history of heart disease, hypertension, diabetes, white matter hyperintensities, depressive symptoms, and time from blood draw to PET imaging acquisition (N = 124 in conservatively adjusted models). However, further adjustment for APOE ε4 status in exploratory, sensitivity models (N = 117) attenuated regional interaction effects in the anterior cingulate and frontal cortex, although results were trending (anterior cingulate gyrus: $\beta = -1.312$ $p = 0.076$; frontal cortex: $\beta = -1.418$; $p = 0.052$).

The interactions between IL-6 and hippocampal volume on PiB retention globally ($p = 0.047$; $\beta = -.173$; R^2 change = 0.027) and in the precuneus ROI ($p = 0.024$; $\beta = -0.195$; R^2 change = 0.037) remained in the CN subsample, but was trending and no longer significant in the anterior cingulate ($p = 0.066$; $\beta = -.160$; R^2 change = 0.025) and frontal cortex ROI's ($p = 0.068$; $\beta = -.159$; R^2 change = 0.025). The interaction terms withstood correction for demographic factors as well as history of heart disease, hypertension, diabetes, white matter hyperintensities, depressive symptoms and APOE ε4 carrier status (N = 117). Furthermore, the interaction term reached significance in the frontal cortex after adjusting for the aforementioned nuisance variables ($p = 0.038$; $\beta = -.188$; R^2 change = 0.032). Parameter estimates for main effect and interaction terms for models including IL-6 and TNF-α are included in Tables 7 and 8. For visualization purposes,

results from the simple slope analyses predicting global PiB retention are depicted in Figure 6 and Figure 7.

Including interaction terms in statistical models explained an additional 2.5-5% of the variance in global and regional PiB deposition in the CN subsample. Similar to the combined sample, APOE genotype explained the largest amount of variance in global PiB uptake (11.8%), followed by depressive symptoms (4.9%), with the interaction term accounting for the third largest amount of variance in conservatively adjusted models, explaining between 2.9% (IL-6) - 3.6% (TNF-α) of the variance above and beyond all other predictors (total variance in global PiB retention explained = 20.1%).

Table 7. Main effects of IL-6 and hippocampal volume on global and regional PiB uptake, as well as their interaction terms (IL-6 X hippocampal volume), in the combined baseline sample (top section) and in the CN subset (bottom section).

N = 173	**Global PiB**	**Anterior Cingulate Gyrus**	**Frontal Cortex**	**Precuneus**
Combined Sample (MCI & CN) N = 173				
IL-6	P = 0.226; β = 0.094	P = 0.182; β = 0.103	P = 0.178; β = 0.105	P = 0.214; β = 0.096
Hippocampal Volume/ICV	P = 0.135; β = -0.121	P = 0.191; β = -0.105	P = 0.152; β = -0.116	**P = 0.043;** **β = -0.162**
IL-6 X Hippocampal Volume	**P = 0.012;** **β = -0.192** **R^2 change = 0.036**	**P = 0.015;** **β = -0.184** **R^2 change = 0.033**	**P = 0.020;** **β = -0.177** **R^2 change = 0.031**	**P = 0.009;** **β = -0.196** **R^2 change = 0.038**
Cognitively Healthy Subjects (MCI subjects excluded) N = 139				
N = 139	**Global PiB**	**Anterior Cingulate Gyrus**	**Frontal Cortex**	**Precuneus**
IL-6	P = 0.667; β = 0.038	P = 0.603; β = 0.046	P = 0.540; β = 0.054	P = 0.646; β = 0.040
Hippocampal Volume/ICV	P = 0.210; β = -0.115	P = 0.305; β = -0.094	P = 0.194; β = -0.118	P = 0.086; β = -0.156
IL-6 X Hippocampal Volume	**P = 0.047;** **β = -0.173** **R^2 change = 0.029**	P = 0.066; β = -0.160 R^2 change = 0.025	P = 0.068; β = -0.159 R^2 change = 0.025	**P = 0.024;** **β = -0.195** **R^2 change = 0.037**

Bolded values significant at $p < 0.05$. All models adjusting for age, gender, and years of education.

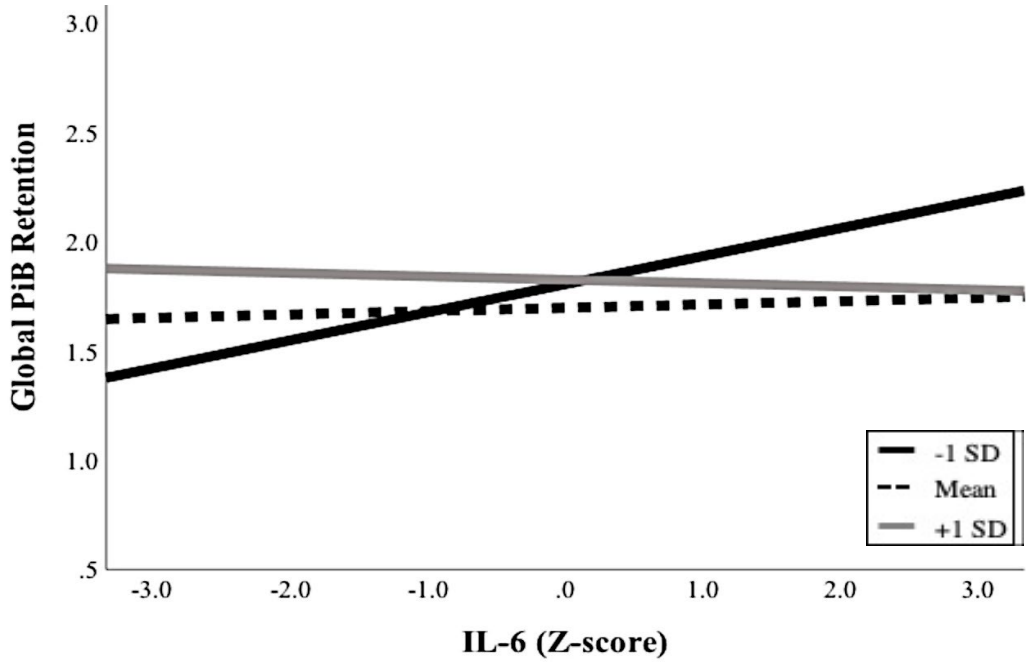

Figure 6. Results from simple slope analysis in the CN subsample (N = 139), illustrating the linear associations between IL-6 and global PiB retention across several conditional values of hippocampal volume.

Table 8. Main effects of TNF-α and hippocampal volume on global and regional PiB uptake, as well as their interaction terms (TNF X hippocampal volume), in the combined sample (top section) and in the CN subset (bottom section).

N = 173	**Global PiB**	**Anterior Cingulate Gyrus**	**Frontal Cortex**	**Precuneus**
Combined Sample (MCI & Cognitively Healthy) N = 173				
TNF-*α*	P = 0.235; β = 0.098	P = 0.337; β = 0.079	P = 0.318; β = 0.083	P = 0.225; β = 0.099
Hippocampal Volume/ICV	P = 0.107; β = -0.130	P = 0.151; β = -0.115	P = 0.118; β = -0.126	**P = 0.032;** **β = -0.171**
TNF-α X Hippocampal Volume	**P = 0.024;** **β = -.172** **R^2 change = 0.029**	**P = 0.027;** **β = -.169** **R^2 change = 0.028**	**P = 0.033;** **β = -.163** **R^2 change = 0.026**	**P = 0.027;** **β = -.168** **R^2 change = 0.028**
Cognitively Healthy Subjects ONLY (MCI subjects excluded) N = 139				
N = 139	**Global PiB**	**Anterior Cingulate Gyrus**	**Frontal Cortex**	**Precuneus**
TNF-*α*	P = 0.643; β = 0.043	P = 0.884; β = 0.014	P = 0.722; β = 0.033	P = 0.411; β = 0.076
Hippocampal Volume/ICV	P = 0.196; β = -0.118	P = 0.288; β = -0.097	P = 0.178; β = -0.123	P = 0.077; β = -0.160
TNF-α X Hippocampal Volume	**P = 0.027;** **β = -.191** **R^2 change = 0.036**	**P = 0.040;** **β = -.178** **R^2 change = 0.031**	**P = 0.039;** **β = -.178** **R^2 change = 0.031**	**P = 0.010;** **β = -.220** **R^2 change = 0.048**

Bolded values significant at $p < 0.05$. All models adjusting for age, gender, and years of education.

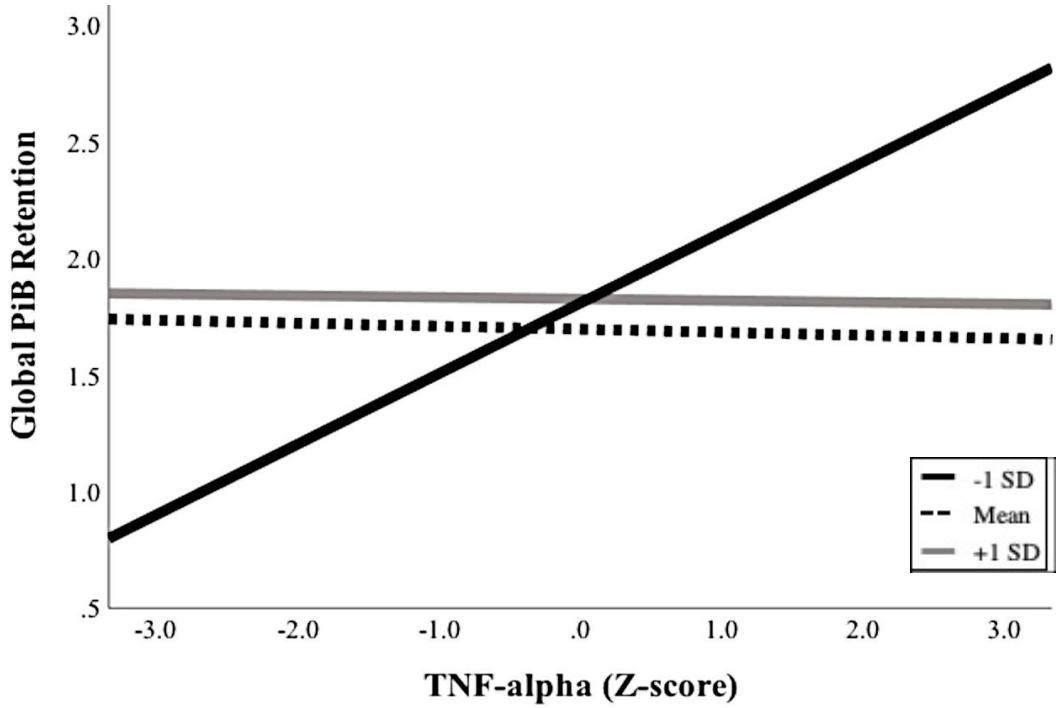

Figure 7. Results from simple slope analysis in the CN subsample, illustrating the linear associations between TNF-α and global PiB retention across several conditional values of hippocampal volume.

3.1.5 Cross-sectional results: summary:

3.1.5.1 Cross-sectional results summary: combined sample

173 subjects completed a blood draw, neuropsychological assessment, and PiB-PET imaging at baseline, including 139 CN subjects and 34 with MCI. The 3 inflammatory biomarkers were not associated with baseline diagnostic status. However, there was an inverse association between concentrations of IL-6 and continuous composite measures of global and verbal memory performance, while no relationship between memory performance and the remaining inflammatory mediators was observed.

We also explored the independent and interactive effects of inflammatory biomarkers and hippocampal volume, an established biomarker of neurodegeneration, on amyloid deposition.

After adjusting for age, gender, and years of education, elevated levels of circulating CD14 were associated with greater Aβ deposition globally and in the anterior cingulate, frontal cortex, and mesial temporal lobe ROI's. Linear regression models revealed no main effect of IL-6 or TNF-α on global PiB-PET retention, precluding secondary regional analyses.

However, we found that hippocampal volume moderated the association between both IL-6 and TNF-α and amyloid load, such that elevated baseline levels of each inflammatory marker were associated with greater global amyloid burden specifically among those with greater hippocampal atrophy. Secondary analyses on regional amyloid deposition revealed that this synergistic relationship was also apparent in the anterior cingulate gyrus, frontal cortex, and precuneus. Notably, for both IL-6 and TNF-α, interaction terms largely withstood adjustment for demographic factors, as well as history of heart disease, hypertension, diabetes, self-reported depressive symptoms, white matter hyperintensities, APOE ε4 carrier status, and time from blood draw to PiB-PET acquisition. In fact, aside from APOE, which has a well-established relationship with amyloid burden, and depressive symptoms, interaction terms were the strongest predictors of PiB retention in hierarchical models. Interaction effects in the occipital lobe and sensory motor cortex did not survive correction for multiple comparisons, and the main effects of CD14 were attenuated after controlling for the aforementioned covariates.

3.1.5.2 Cross-sectional results summary: cognitively healthy subsample

In order to restrict our analyses to those that may be in the preclinical phase of AD, we also explored these relationships exclusively among subjects considered cognitively healthy at baseline (N = 139). In contrast to the null results observed in the combined baseline sample of 173 participants, there was a negative association between TNF-α and verbal memory performance.

Similarly, after holding demographic factors constant, lower circulating levels of IL-6 were associated with better performance on composite measures of global and verbal memory.

With respect to amyloid pathology, excluding the 34 subjects diagnosed with MCI diminished many of the main effects of CD14 observed in the full combined sample. While the relationship between CD14 and PiB retention in the anterior cingulate gyrus persisted, associations with global amyloid load and regional tracer uptake in the frontal cortex and mesial temporal lobe were no longer significant. Conversely, consistent with the findings in the combined sample, neither TNF-α nor IL-6 were independently associated with global amyloid deposition. There was also no main effect of hippocampal volume on cortical amyloid load. However, moderation models revealed that the relationship between both biomarkers and amyloid pathology varied as a function of hippocampal degeneration. Specifically, among individuals with greater hippocampal atrophy, elevated peripheral concentrations of TNF-α were predictive of higher PiB retention globally and in several regions of interest, including the anterior cingulate gyrus, frontal cortex, and precuneus. Notably, these interaction effects persisted after further adjustment for history of heart disease, diabetes, hypertension, white matter lesions, depressive symptoms, and time from blood draw to imaging. Adding APOE status to the model weakened interaction effects in the anterior cingulate gyrus and frontal cortex, resulting in trending associations for both ROIs. Hippocampal volume also moderated the association between IL-6 and PiB retention globally and in the precuneus, even after adjustment for demographic factors, cardiovascular risk factors, depression, and APOE status. Interaction terms were trending for the anterior cingulate gyrus, and reached significance in the frontal cortex ROI in conservatively adjusted models.

3.2 LONGITUDINAL RESULTS

3.2.1 Subject characteristics

Of the 173 participants that had neuropsychological, neuroimaging, and plasma data at baseline, 90 participants completed follow-up assessments 2 years later. The average age of the 90 participants included in the longitudinal analyses was 85.40 (SD = 2.929), with ages ranging from 82-95 at baseline. Women comprised 41.1% of the sample (N = 37), all but 4 subjects were Caucasian (95.6), and participants had an average of 14.78 years of education (SD = 2.77). Forty-nine subjects were categorized as Aβ- at baseline (54.4%), and 41 (45.6%) were considered Aβ+. Fifteen participants were diagnosed with MCI at baseline (16.7%), including 6 with amnestic MCI and 9 with non-amnestic MCI. Of the full sample, 13 subjects (15.3%) were carriers of the APOE ε4 genotype. Of the 75 participants that were cognitively healthy at baseline, 20 received a diagnosis of MCI at follow-up. According to a series of Chi-squared and two-tailed t-tests, the 83 subjects that did not undergo neuroimaging at T_2 did not differ from the 90 participants that returned for follow-up in terms of age, education, and concentrations of inflammatory biomarkers. There was a significant group difference in global and regional PiB retention, such that participants that did not return for follow-up, on average, had higher levels of baseline amyloid deposition relative to those included in the longitudinal sample.

Among the 90 subjects included in the longitudinal sample, the 15 subjects with MCI did not differ from the 75 CN subjects in terms of gender and APOE ε4 distributions, age, years of education, or self-reported depressive symptoms. Those with MCI, on average, had significantly lower MMSE scores than the 75 CN subjects, although that is to be expected ($t = 3.17$; $p = 0.032$). Two-tailed bivariate correlations demonstrated a moderate correlation between TNF-α and CD14

in the full sample (r = 0.214, p = 0.043), but not IL-6 (r = 0.092; p = 0.0388). CD14 was significantly correlated with IL-6 (r = 0.223; p = 0.035). Table 9 includes descriptive characteristics for the combined longitudinal sample and the CN and MCI subsets.

Table 9. Descriptive statistics for combined T1-T2 longitudinal sample and CN and MCI subsamples.

	Combined Sample N = 90	**CN Subsample** N = 75	**MCI** N = 15
	% (N) or *M (SD)*	% (N) or *M (SD)*	% (N) or *M (SD)*
Age	85.4 (2.93)	85.31 (2.98)	85.87 (2.7)
Gender, % F (N)	41.1 (37)	45.3 (34)	20 (3)
Education	14.78 (2.77)	14.99 (2.77)	13.73 (2.63)
ApoE, % (N)	14.4 (13)	17.3 (13)	93.3 (14)
Hx Heart Disease, % (N)	18.9 (17)	14.7 (11)	40 (6)
Diabetes, % (N)	5.6 (5)	4 (3)	13.3 (2)
Hypertension, % (N)	27.8 (25)	25.3 (19)	40 (6)
MCI, % (N)	16.7 (15)	--	--
MMSE	27.85 (1.93)	28.13 (1.65)	26.43 (2.59)
CD14	1374.64 (286.78)	1380.92 (279.78)	1343.21 (328.31)
TNF-α	3796.11 (960.14)	3716.30 (924.33)	4195.18 (1067.56)
IL-6	2.76 (1.99)	2.66 (1.76)	3.27 (2.94)
Weeks from blood draw to imaging	180.89 (36.58); range 125 - 423	180.3 (38); range 125 - 423	185.2 (21.72); range 146 - 214
T1 PiB+, % (N)	45.6 (41)	40 (30)	73.3 (11)
T2 PiB+, % (N)	58.9 (53)	54.7 (41)	80 (12)
CVLT Immediate	45.89 (11.39)	47.79 (10.90)	36.4 (9.02)
CVLT Delay	8.92 (3.60)	9.57 (3.29)	5.67 (3.42)
Rey-O Immediate	16.79 (3.56)	17.23 (3.53)	14.6 (2.83)

3.2.2 Longitudinal relationships between inflammatory biomarkers, cognitive diagnosis, and memory performance

3.2.2.1 Combined sample

Hierarchical logistic regression models were employed to evaluate the association between baseline inflammatory biomarkers and diagnostic status at two-year follow-up. Of the 90 subjects that completed a neuropsychological assessment and neuroimaging at both time points, 32 met

criteria for MCI and 58 subjects remained cognitively normal at follow-up. After controlling for other risk factors associated with cognitive decline, including age, gender, and years of education, those with elevated baseline concentrations of IL-6 had a higher risk of having a diagnosis of MCI at 24 months (OR (95% CI) = 1.81 (1.08; 3.02), p = 0.024). TNF-α was not significantly associated with diagnostic status (OR (95%) = 1.29 (0.72; 2.31), p = 0.40), and CD14 was trending but non-significant (OR (95% CI) = 1.74 (0.96; 3.14), p = 0.068).

Linear regression models were also employed to evaluate the association between inflammatory biomarkers and longitudinal memory performance. Dependent variables were unstandardized residuals, which reflected change in memory composite scores between the two time points, holding baseline performance constant. Elevated concentrations of TNF-α predicted a subsequent decrease in performance on measures of global memory ($\beta = 0.264$; $p = 0.023$; R^2 change = 0.059) and verbal memory ($\beta = 0.235$; $p = 0.041$; R^2 change = 0.047), but not visuospatial memory. There were no statistically significant associations between baseline CD14 or IL-6 and longitudinal change in memory performance (all $p > 0.05$).

3.2.2.2 CN subsample

To investigate whether inflammatory biomarkers predicted progression from CN to MCI at follow-up, binary logistic regression analyses were performed. Of the 75 subjects that were cognitively healthy at baseline, 20 met diagnostic criteria for MCI at the 24-month follow-up. IL-6 predicted subsequent cognitive status at 24 months, such that the risk of diagnostic conversion over 2 years was greater among those with elevated concentrations of IL-6 at baseline (OR (95% CI) = 1.86 (1.03; 3.31); p = 0.039). CD14 and TNF-α did not predict change in cognitive status at follow-up. Regarding continuous composite measures of memory performance, none of the individual biomarkers predicted change in memory performance over the course of two years (all $p > 0.05$).

Associations between CD14 and global memory performance were trending but non-significant (B = .00; β = 0.219; CI = 0; 0; p = 0.094; R^2 = 0.039). Similarly, the association between TNF-α and change in delayed performance on the CVLT was trending but no longer significantly different from zero in the CN subsample (B = .001; β = 0.249; CI = 0; .001; p = 0.055; R^2 = 0.051).

3.2.3 Longitudinal associations between inflammatory biomarkers, hippocampal volume, and Aβ deposition

3.2.3.1 Combined sample

Multiple linear regression models were conducted to explore associations between inflammatory biomarkers and change in fibrillar amyloid deposition between the two consecutive scans, while adjusting for age, gender, and education level. Dependent variables were residualized change estimates of global and regional PiB retention, and independent variables of interest were inflammatory biomarkers, hippocampal volume, and their interaction products. There were no associations between any of the 3 inflammatory biomarkers on longitudinal change in global or regional amyloid deposition (all p > 0.05). The interaction between each biomarker and baseline hippocampal volume did not predict change in global or regional PiB retention in the combined sample.

3.2.3.2 CN subsample

While IL-6 was not associated with subsequent Aβ accumulation in the combined sample, restricting our analyses to the 75 subjects deemed CN at baseline revealed a main effect of IL-6 on longitudinal change in PiB retention over 24 months. Specifically, elevated baseline concentrations of IL-6 predicted greater regional amyloid accumulation over 2 years in the anterior

cingulate gyrus (β = .272; p = 0.023; R^2 change = 0.080) and precuneus (β = .243; p = 0.041; R^2 change = 0.057). The spatial specificity of these relationships was probed further using hand-drawn ROI's, which demonstrated associations between elevated IL-6 and increased PiB retention in the dorsal precuneus (β = .324; p = 0.006; R^2 change = 0.103) and the superior (β = .236; p = 0.047; R^2 change = 0.058) and pregenual subregions (β = .284; p = 0.017; R^2 change = 0.10) of the anterior cingulate gyrus (see Table 10). IL-6 did not predict longitudinal change in global amyloid deposition (β = 0.158; p = 0.190; R^2 change = 0.028). Results remained when using an alternative change estimation approach, in which Aβ deposition at T_2 served as the dependent variable for each ROI, holding constant PiB retention at T_1. The direct effects of IL-6 on change in regional Aβ accumulation also withstood adjustment for hypertension, depressive symptoms, and white matter hyperintensities at T_2. Aside from the superior anterior cingulate ROI, these associations also survived correction for the duration of time between blood draw to follow-up imaging (mean weeks (SD) = 180.3 (38); range = 125 – 423). After adjusting for all of the aforementioned covariates in hierarchical models, IL-6 accounted for 7.5-12.5% of the variance in change in regional PiB retention across these ROI's (coefficients included in Table 10). Significance was attenuated in anterior cingulate ROI's after controlling for history of heart disease and ApoE ε4 status (Note: n = 69 in conservatively adjusted models). Diabetes was not included as a nuisance variable given the small number of subjects in the longitudinal subsample (N = 3) with diabetes at baseline. Neither TNF-α nor CD14 predicted change in global or regional PiB uptake in the cognitively healthy subsample. For each of the three biomarkers, interaction terms did not reach statistical significance.

Table 10. Parameter estimates from conservatively adjusted hierarchical linear regression models evaluating associations between IL-6 concentrations and change in amyloid deposition in ROIs.

	Anterior Cingulate Gyrus N = 65			**Posterior Anterior Cingulate Gyrus** N = 65		
	β	P-value	R^2 change	β	P-value	R^2 change
Age	-0.011	0.387		-0.044	0.756	
Gender	0.045	0.525		0.049	0.727	
Education	-0.016	0.212		-0.089	0.516	
Blood draw to imaging duration	0.001	0.311		0.161	0.265	
Hypertension	0.03	0.71		0.096	0.503	
WMH	-0.001	0.969		-0.06	0.688	
CES-D	0.004	0.591		0.079	0.562	
Hippocampal Volume	-0.239	0.652	0.072	-0.148	0.28	0.072
IL-6	0.319	0.019*	0.089	0.32	0.016*	0.093
	Precuneus			**Upper Precuneus**		
	β	P-value	R^2 change	β	P-value	R^2 change
Age	-0.279	0.044		-0.227	0.106	
Gender	0.057	0.668		-0.006	0.967	
Education	-0.041	0.755		-0.051	0.702	
Blood draw to imaging duration	0.258	0.063		0.252	0.075	
Hypertension	0.003	0.982		-0.026	0.849	
WMH	0.1	0.481		0.134	0.354	
CES-D	-0.036	0.783		-0.099	0.455	
Hippocampal Volume	0.083	0.525	0.154	0.024	0.859	0.124
IL-6	0.286	0.025*	0.075	0.371	0.004*	0.125

* Significant at $p < 0.05$.

Note: Hippocampal volume was included with IL-6 as a predictor variable in moderation models, however, given the main effects of IL-6 the models were re-run to isolate the amount of variance explained by IL-6 above and beyond all other variables.

3.2.4 Longitudinal summary

3.2.4.1 Longitudinal summary: combined sample

Of the 173 participants that completed baseline PiB-PET imaging and neuropsychological assessments, 90 older adults underwent repeated assessments 24 months later, including 75 subjects that were CN at T_1 and 15 that met diagnostic criteria for MCI. This prospective data was

used to evaluate associations between baseline concentrations of circulating peripheral inflammatory biomarkers and longitudinal changes in cognition and amyloid pathology. After accounting for demographic factors, subjects with elevated baseline concentrations of IL-6 were more likely to carry a diagnosis of MCI at the 24-month follow-up. Neither TNF-α nor CD14 were predictive of diagnostic status. Linear models including continuous estimates of memory performance revealed that elevated levels of circulating TNF-α predicted worse longitudinal performance on a composite measure of global memory. Probing this relationship using task-specific composites revealed that this was largely driven by an association between higher baseline TNF-α and subsequent declines in verbal memory performance. Despite the relationship between IL-6 and diagnostic status, this biomarker did not predict change in memory performance.

Linear models investigating the association between inflammatory biomarkers and prospective Aβ accumulation revealed no significant direct effects of inflammatory mediators on change in global or regional amyloid deposition over two years. In contrast to the cross-sectional findings using the combined baseline sample (N = 173), baseline hippocampal volume did not moderate the relationship between inflammatory markers and longitudinal amyloid accumulation.

3.2.4.2 Longitudinal summary: CN subsample

Among the 75 subjects that were cognitively healthy at baseline, logistic regression models revealed that elevated concentrations of IL-6 at T_1 conferred a higher risk of conversion to MCI at 24-months. There was not a significant relationship between CD14 or TNF-α and subsequent diagnostic conversion. TNF-α no longer predicted change in memory performance after excluding the 15 subjects with MCI, and associations between CD14 and IL-6 and continuous measures of memory performance remained non-significant.

In contrast to the null findings observed in the combined sample, restricting the analyses to subjects that were cognitively healthy at baseline revealed significant positive associations between high baseline concentrations of IL-6 and longitudinal Aβ accumulation over the course of two years. Exploration of hand-drawn ROIs revealed these associations to be regionally specific, such that IL-6 predicted increased PiB retention in the precuneus (globally and specifically in the upper precuneus) and the anterior cingulate gyrus (including both the pregenual and superior anterior cingulate regions). Adding IL-6 to models adjusting for demographic characteristics accounted for an additional 5.7-10.3% of the variance in progression of amyloid pathology over 24 months. There was no association with change in global PiB retention, nor was there an interaction between baseline hippocampal volume and IL-6. However, IL-6 remained a significant independent predictor of change in amyloid deposition after controlling for hypertension, white matter hyperintensities, and self-reported depressive symptoms, but attenuated in all ROI's aside from the upper precuneus after additional adjustment for history of heart disease and APOE ε4 genotype. Taken together, higher concentrations of circulating IL-6 predicted subsequent conversion to MCI and increased longitudinal accumulation of Aβ pathology in regions susceptible to early amyloid deposition exclusively among participants considered cognitively healthy at baseline. There were no direct effects of CD14 or TNF-α on change in amyloid burden, and interaction terms failed to reach statistical significance.

4.0 DISCUSSION

4.1 SUMMARY

Individuals harbor Aβ plaques for 10-20 years before the symptoms of AD become clinically apparent. In order to delay or even prevent AD, the greatest chance for success likely involves intervening *early* in this pathogenic process, well before the onset of cognitive symptoms. Offsetting this disease trajectory requires an enhanced understanding of the molecular processes that initiate and exacerbate Aβ prior to symptom manifestation. Abundant evidence from rodent and primate models demonstrates the central contribution of pro-inflammatory processes to the generation and progression of neurotoxic Aβ. Despite this, remarkably little translational work has been conducted in humans. The present study addressed substantial gaps in the literature by exploring the association between peripheral inflammatory markers, cognition, and amyloid pathology in a sample of non-demented elderly adults. Using the NIA-AA framework of preclinical AD, this study also aimed to evaluate potential disease-state-dependent differences in these relationships specifically among CN older adults. Finally, for the first time in humans, we determined whether peripheral inflammatory biomarkers predicted the longitudinal progression of amyloid pathology during this critical preclinical phase. Understanding how these biomarkers are associated with AD-related pathology prior to the onset of cognitive symptoms could help identify individuals at high risk for impending cognitive decline, improve timely diagnoses of AD, and facilitate earlier intervention.

In a sample of 173 non-demented elderly adults, we found that indicators of chronic, systemic inflammation were associated with and predictive of the progression of one of the

primary neuropathologic features of AD. Specifically, higher circulating concentrations of two peripheral pro-inflammatory biomarkers, IL-6 and TNF-α, were associated with elevated global Aβ deposition specifically among those that also exhibited greater hippocampal atrophy. Secondary ROI analyses demonstrated that interaction effects between inflammatory biomarkers and hippocampal volume were specific to PiB uptake in regions susceptible to early Aβ accumulation, including the precuneus, anterior cingulate gyrus, and prefrontal cortex. Both TNF-α and IL-6 were also independently associated with poorer verbal memory performance in CN older adults. Finally, longitudinal analyses revealed that elevated baseline concentrations of IL-6 predicted conversion to MCI and greater regional amyloid accumulation over the course of 2 years.

4.2 CROSS-SECTIONAL FINDINGS

4.2.1 Inflammatory biomarkers and cognitive performance

Extant studies in humans have largely evaluated the relationship between inflammation and AD by comparing concentrations of inflammatory biomarkers between individuals with and without detectable cognitive impairment. This includes epidemiological studies that demonstrate differences in cytokine expression between cognitively healthy older adults and those with advanced AD (Brosseron et al., 2014). However, the relationship between inflammatory markers and subtle cognitive changes prior to dementia onset has not been well characterized (Brosseron, Krauthausen, Kummer, & Heneka, 2014; Saleem, Herrmann, Swardfager, Eisen, & Lanctot, 2015). To decompose this, we examined the relationship between peripheral biomarkers and

cognition in a large sample of non-demented older adults (MCI & CN), and assessed whether associations were specific to, or observed exclusively in, cognitively intact older adults.

We found that elevated levels of IL-6, an indicator of chronic, systemic inflammation, were associated with poorer global and verbal memory performance in the full sample. Moreover, the relationship between IL-6 and verbal memory persisted after restricting the analysis to CN participants. This is consistent with findings from Marsland et al., (2015) showing that higher concentrations of circulating IL-6 were associated with poorer performance on measures of spatial reasoning, learning, memory, and executive function in a sample of middle-aged adults (Marsland et al., 2015). However, although studies in CN older adults demonstrate associations between IL-6, executive function, and processing speed, they have failed to find links with immediate or delayed verbal memory performance (Heringa et al., 2014; Palta et al., 2014; Stenfors, Jonsdottir, Hanson, & Theorell, 2017; Tegeler et al., 2016). The discrepancies between the present findings and prior work in older adult populations may be due to variations in the verbal memory measures used, small sample sizes, and the age distribution of study populations. The age range of the GEMS-PGG sample is more advanced than those included in the aforementioned studies. While chronic, low-grade inflammation may initially be associated with delayed processing speed and executive dysfunction in older adulthood, it's possible that these relationships become more broadly distributed and encompass multiple cognitive domains with advancing age. The availability of processing speed and executive function measures in this dataset offers opportunities for further evaluation of the domain-specificity of this relationship in this sample.

Interestingly, associations between TNF-α and verbal memory performance surfaced only after excluding subjects with MCI. The specificity of this relationship in the CN subsample suggests potential disease-state-dependent differences in biomarker expression, such that elevated

TNF-α may be associated with subtle cognitive changes prior to the onset of clinically detectable cognitive symptoms. This could account for some of the discrepant findings across studies evaluating differences in TNF-α levels between CN, MCI, and early AD populations. For instance, a recent meta-analysis found that 2 studies reported up-regulation of TNF-α in the plasma or CSF of patients with MCI relative to controls, while 3 showed no group differences and one reported downregulation of TNF-α among those with MCI (Brosseron et al., 2014). It is becoming increasingly clear that these inflammatory processes become relevant much earlier in the disease course, well before symptom manifestation. Accordingly, those in the preclinical phase may already be expressing elevated inflammatory levels without exhibiting outward cognitive deficits, thereby diluting potential group differences in biomarker expression between CN and MCI populations.

4.2.2 Inflammatory biomarkers, neurodegeneration, and amyloid pathology

4.2.2.1 Disease state-dependent differences in biomarker expression

Pro-inflammatory states are now widely recognized as naturally occurring and predominant features of nearly all neurodegenerative diseases, including AD. Nevertheless, how these inflammatory processes impact the pathogenesis of AD, and when they first start to take effect, is poorly understood. We aimed not only to characterize the relationship between inflammatory biomarkers and amyloid pathology in non-demented subjects, but also to explore how these relationships may vary across different stages along the preclinical continuum of AD. According to the NIA-AA, amyloid deposition is the first detectable change in the preclinical cascade, followed subsequently by synaptic dysfunction, hippocampal atrophy, and the formation of neurofibrillary tangles (phase 2), which develop prior to the onset of cognitive symptoms (Counts

et al., 2016; Jack et al., 2011; R. Sperling et al., 2014; Vlassenko et al., 2012). In order to distinguish individuals potentially representing more advanced stages of preclinical disease progression, hippocampal volume was used in the present study as a proxy for neurodegeneration.

Consistent with our hypothesis, the association between biomarker expression and Aβ varied as a function of hippocampal volume, such that higher levels of IL-6 and TNF-α were each predictive of elevated amyloid deposition specifically among those exhibiting greater neurodegeneration. In other words, while neither hippocampal volume nor TNF-α or IL-6 were independently associated with PiB uptake, their combined presence was associated with greater global amyloid burden. Simple slope analyses revealed that among subjects with average to high hippocampal volume (e.g., displaying minimal hippocampal degeneration), amyloid deposition remained stable across varying levels of cytokine expression. Conversely, among individuals exhibiting the most hippocampal atrophy, low levels of TNF-α and IL-6 were associated with reduced amyloid load, whereas elevated concentrations predicted greater amyloid burden. These findings were observed in the full sample and persisted after restricting our analyses to the CN subsample, indicating that the subjects with MCI did not drive these significant interaction effects.

The process of microglial priming following early Aβ aggregation lends mechanistic support to the interaction effects observed in the present study. While the cumulative impact of inflammatory processes appears detrimental, the pro-inflammatory cascade may, temporarily, be neuroprotective. Indeed, rodent models indicate that microglial activation initially helps to reduce Aβ pathology by increasing phagocytosis, promoting the breakdown and clearance of neurotoxic Aβ species, and stimulating the release of protective trophic factors (Fan et al., 2015; Heneka et al., 2015; Ramesh et al., 2013; W.-Y. Wang et al., 2015). However, chronic or prolonged exposure to amyloid aggregates results in microglial priming, causing these highly plastic immune cells to

become highly sensitized to further activation. This results in a sustained, pro-inflammatory environment characterized by the overexpression of chemokines and cytokines, including IL-6 and TNF-α. In the present study, we attempted to approximate or estimate this phenotypic shift by identifying those exhibiting downstream neurodegenerative changes in the form of hippocampal atrophy. Participants with greater hippocampal atrophy may be further along the preclinical continuum, suggesting prolonged exposure of brain-resident immune cells to neurotoxic Aβ species and, potentially, an enhanced sensitivity to peripheral pro-inflammatory signaling.

Although there are numerous pathways by which this pro-inflammatory cascade potentiates the generation of Aβ species, of particular relevance to the present study are animal models elucidating the direct effects of IL-6 and TNF-α on Aβ. For example, over-expression of both IL-6 and TNF-α up-regulate the aberrant cleavage of APP (Calsolaro & Edison, 2016; Walters et al., 2016), which is necessary for Aβ generation. TNF-α signaling also increases the expression of β-secretase and the activity of γ-secretase (Calsolaro & Edison, 2016; Sastre et al., 2003; Walters et al., 2016), the cleaving enzymes that produce neurotoxic Aβ (Heppner et al., 2015). Notably, introducing anti-inflammatory agents that target these pathways lowers Aβ generation and β-secretase activity, abolishes Aβ-related cellular toxicity, and improves learning and memory deficits in rodent and primate models (Decourt et al., 2016; He et al., 2013); (Shamim & Laskowski, 2017; Tweedie et al., 2012). Both TNF-α and IL-6 exacerbate the pro-inflammatory drive in the CNS by enabling further activation of primed microglial cells (Calsolaro & Edison, 2016; W.-Y. Wang et al., 2015).

While not specific to the preclinical phase, other work in humans supports the possibility of disease-state-dependent differences in inflammatory profiles. For instance, in a cross-sectional study by Parbo and colleagues (2018), 6 subjects with AD and 20 with MCI underwent

inflammation (^{11}C-(R)-PK11195), amyloid (^{11}C-PiB), and tau (^{18}F-flortaucipir) PET imaging. The authors observed correlations between microglial activation and amyloid deposition in individuals with amnestic MCI, but not those diagnosed with AD. Furthermore, they found elevated microglial activation in PiB+ MCI cases where tau signals were low. The authors concluded that the correspondence between inflammatory processes and Aβ deposition may be stronger prior to AD onset. They also noted that immune cell activation may precede tau deposition, and thus represent an important process that occurs earlier in the disease course (Parbo et al., 2018). Similarly, Yasuno et al., (2012) found elevated microglial tracer uptake among subjects with MCI and AD relative to controls, but no difference in microglial activation between those with MCI and AD (Yasuno et al., 2012). The absence of group differences among those with MCI and AD suggests that particular inflammatory processes may be elevated earlier in the disease course**.** There are several implications of these findings, including the possibility that there may be critical periods when amyloid lowering therapies targeting inflammatory processes may be most efficacious.

One major caveat of this interpretation is that hippocampal degeneration in older adulthood is not specific to AD. Hippocampal atrophy can develop in the context of many other neuropathological conditions, including Lewy body disease, medial temporal tauopathy without Aβ, and cerebrovascular disease (Jack et al., 2014). Sometimes termed suspected non-Alzheimer pathophysiology (SNAP), this reflects populations that exhibit abnormal imaging biomarker(s) but do not fit into the NIA-AA framework due to an absence of elevated Aβ. A seminal study found that 23% of participants with biomarker abnormalities aligning with AD-related pathology (e.g., hippocampal degeneration, hypometabolism) fit into the SNAP group (Jack et al., 2014), which is consistent with more recent estimates (Morimono et al., 2016). This is an important limitation to the cross-sectional interpretation, as it cannot be assumed that reduced hippocampal volume is

indicative of an underlying or ongoing AD process (or even progression along the preclinical AD continuum). It is possible that those who exhibit greater hippocampal degeneration are generally more sensitive to peripheral influences, although this may not be due to an underlying AD process.

To try to address this limitation, we conducted a *post hoc* analysis exploring the interaction between inflammatory biomarkers and hippocampal volume exclusively among the 93 subjects that exhibited elevated global amyloid deposition based on conventional dichotomous standards. Therefore, greater hippocampal atrophy among these PiB+ participants may better reflect the latter stages of neuropathological progression in preclinical AD. Notably, the interaction between IL-6 and hippocampal volume persisted in this subsample, indicating that among subjects meeting conventional thresholds of PiB positivity, elevated concentrations of IL-6 were associated with greater global amyloid burden among those also displaying hippocampal atrophy. Notably, the effect size of the interaction term nearly doubled after restricting the analysis to PiB+ subjects.

4.2.2.2 Direct associations between inflammatory biomarkers, hippocampal atrophy, and Aβ deposition

Although the bulk of the cross-sectional findings involved TNF-α and IL-6, main effects models revealed a direct association between elevated soluble CD14 concentrations and greater global amyloid deposition at baseline. CD14 is a surface antigen that is abundantly expressed on monocytes and macrophages in the periphery. CD14 detects invading pathogens and in particular binds to lipopolysaccharide (Zhan et al., 2018), interacting with toll-like receptors to facilitate phagocytosis and induce the production of several cytokines including IL-1, TNF-α, and IL-6 (Leveque, Jeune, Jouneau et al., 2017). Relative to TNF-α and IL-6, CD14 has been understudied in humans and rodent models of AD. Therefore, the precise mechanisms linking this cell surface receptor to amyloid pathology remain speculative. It is notable that the direct association between

CD14 and amyloid load did not survive adjustment for cardiovascular risk factors in the present study. Furthermore, two-tailed T tests also showed that CD14 levels were significantly higher in hypertensive relative to normotensive subjects, with trending group differences among those with/without a history of cardiovascular disease. Statin treatment among those with hypertension reduces soluble CD14 expression (Frey et al., 2007). CD14 may also be involved in low-density lipoprotein binding and has been shown to promote low-density lipoprotein-induced cytokine release (Estruch, Bancells, Beloki et al., 2013). Taken together, the cross-sectional associations with amyloid load observed here may be linked to the relationship between CD14 and cardiovascular risk factors.

Main effects models also revealed that hippocampal atrophy did not independently predict global PiB retention. The absence of a main effect of hippocampal volume on Aβ deposition found here is consistent with numerous prospective and cross-sectional studies demonstrating no association between amyloid load and hippocampal atrophy at the earliest stages of disease onset (Jack et al., 2014; Vos et al., 2013 Achenbrenner et al., 2018). In fact, these findings have been used to inform and confirm the NIA-AA framework of amyloid pathogenesis as a biologically distinct process that is initiated prior to AD-related neurodegeneration.

4.2.2.3 Regionally specific associations between inflammation, hippocampal volume, and amyloid deposition

Of the circumscribed body of literature that has explored the relationship between peripheral inflammation and amyloid pathology, most has been conducted using CSF-derived measures of Aβ. This is the second study, to our knowledge, to investigate the associations between peripheral pro-inflammatory biomarkers and amyloid pathology measured in the brain, and the first to explore the regional specificity of these relationships. We found that both TNF-α and IL-6 were associated

with focal Aβ deposition in the anterior cingulate gyrus, frontal cortex, and precuneus ROIs. Notably, hierarchical staging models of preclinical Aβ progression indicate that these regions are associated with deposition patterns that occur during the earliest stages of amyloidosis (Cho et al., 2016; Vlassenko et al., 2012; Grothe et al., 2017; Villeneuve et al., 2015). Interaction effects were also apparent in the mesial temporal lobe and occipital structures, although these did not meet multiple comparison correction thresholds. These trends align with extant studies in older adults showing regionally distinct relationships between systemic inflammation and brain structure and function. For instance, in a sample of cognitively healthy older adults, Warren and colleagues (2018) found that elevated levels of IL-6 predicted greater declines in cerebral blood flow specifically in the orbitofrontal cortex and hippocampal region over a 5-year period (Warren et al., 2018). In a middle-aged sample, Marsland et al., (2017) found that IL-6 was associated with alterations in functional connectivity in the anterior cingulate and dorsal medial prefrontal regions of the Default Mode Network (Marsland et al., 2017). Dev and colleagues (2017) also found that elevated IL-6 predicted reduced BOLD activation in the parietal cortex and middle frontal gyrus during a working memory task (Dev et al., 2017). Similarly, elevated TNF-α levels have been linked to reduced gray matter volume in the medial prefrontal cortex and hippocampus in middle-aged (Marsland et al., 2015) and older adults (Zhang et al., 2016), as well as decreased functional connectivity in the default mode network (Magalhães et al., 2018). Taken together, these studies provide evidence of the relationship between systemic inflammation and variations in cerebral blood flow, neural recruitment, and functional connectivity in the prefrontal, mesial temporal, and parietal cortices. Along with corroborating the regional specificity observed in the present work, these studies also illustrate the sensitivity of the aging brain to peripheral inflammatory states.

4.3 LONGITUDINAL FINDINGS

4.3.1 Systemic inflammation and AD: longitudinal relationships between peripheral biomarkers, cognition, and amyloid pathology

The exploration of the role of peripheral inflammatory biomarkers in the present study reflects an evolving perspective of AD pathogenesis as a systemic process. Novel perspectives of AD etiology have begun to consider the entire system, including the critical impact of peripheral health, on disease onset and progression (Eikelenboom et al., 2012; Mietelska-Porowska & Wojda, 2017; J. Wang, Gu, Masters, & Wang, 2017). This is exemplified by a recent review in *Nature Reviews, Neuroscience*, in which the authors state, "We propose that AD might not be solely a brain disorder, in the sense that systemic factors might interact with the brain to modify the AD process… The crucial questions to answer would be precisely how the brain and periphery interact with each other to affect AD progression, and whether interventions that target systemic factors can modulate the pathogenesis or development of AD" (Wang et al., 2017). Identifying systemic factors that contribute to the pathogenesis of AD could offer a host of new therapeutic opportunities targeting peripheral health to alter disease risk (Wang et al., 2017).

Consistent with this notion, results from the present study reveal relationships between estimates of chronic inflammation measured in the systemic circulation and AD-related pathology in the brain. Mechanistically, this is supported by work showing that primed microglia exhibit an exaggerated response to both central and peripheral inflammatory signaling. While it was once believed that inflammatory cells in the circulation could not invade the CNS, it is now understood that complex, dynamic, and elaborate pathways exist that allow for peripheral immune cells to infiltrate the CNS and influence inflammatory processes in the brain (see Capuron and Miller,

2011, for review). In the context of AD, overexpression of pro-inflammatory cytokines following initial Aβ deposition functionally alters the permeability of the BBB, reducing the integrity of this critical gating system and enabling the infiltration of peripheral immune cells into the brain ((Bradburn et al., 2018; Calsolaro & Edison, 2016; Heppner et al., 2015; MacPherson et al., 2017; W.-Y. Wang et al., 2015). Indeed, acute systemic events restricted to the periphery, such as induced osteoarthritis, increase pro-inflammatory signaling in the brain, as well as Aβ aggregation and deposition in rodents (Kyrkanides et al., 2011). Moreover, introducing inhibitors of these pro-inflammatory pathways into the systemic circulation reduces microglial activation and decreases the deposition of Aβ peptides (MacPherson et al., 2017). Thus, the early deposition of Aβ alters local immune cell signaling, permitting pro-inflammatory mediators that proliferate in a state of chronic inflammation to have additive or even distinct neuromodulatory effects.

This dynamic immune-brain communication in the context of AD has given rise to the 'second hit' hypothesis**,** suggesting that those with Aβ pathology are particularly susceptible to subsequent inflammatory events, including those that originate in the periphery. This theory is supported, in part, by studies showing that acute systemic inflammatory events can result in a combination of symptoms known as 'sickness behavior' among individuals with AD. Often induced by infection, injury, or other immune-compromising conditions (e.g., respiratory infections, genitourinary infections, surgical interventions), sickness behavior reflects a constellation of neuropsychiatric symptoms, including anxiety, depressed mood, apathy, social withdrawal, listlessness and fatigue (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; C Holmes, Cunningham, Zotova, Culliford, & Perry, 2011; Maes et al., 2012). In a prospective cohort study of 300 older adults with AD, raised serum concentrations of both TNF-α and IL-6 predicted a 2-fold increase in the frequency of neuropsychiatric symptoms associated with sickness

behavior, including low mood, agitation, and anxiety (Holmes et al., 2011). Furthermore, the rate of cognitive decline doubled among subjects that experienced acute systemic inflammatory events over the 6-month follow-up period (Holmes et al., 2009). Moreover, acute illness and raised levels of pro-inflammatory markers increase the likelihood of delirium onset among those with AD (Simone & Tan, 2011). Taken together, these results demonstrate that, in this highly vulnerable population, more severe or acute systemic inflammatory events lead to significant alterations in symptom presentation and severity. This work also provides corroborating evidence of the mediating role of IL -6 and TNF-α in facilitating communication between the periphery and the brain.

Our longitudinal results support and expand upon this work by showing that along with acute events, sustained, low-grade chronic inflammation may also have neural and cognitive consequences. The findings in the present study further indicate that this systemic inflammatory state may become relevant much earlier in the disease course, leading to more subtle changes in cognitive function and potentiating Aβ pathogenesis**.** Specifically, we found that elevated IL-6 levels predicted a regionally specific pattern of Aβ progression, including increased amyloid deposition in sub-regions of the anterior cingulate gyrus and precuneus. These results are consistent with the regionally distinct findings observed in our cross-sectional models, and reflect brain areas that are most vulnerable to early Aβ deposition. A critical facilitator of both the acute phase response and sustained inflammatory signaling, IL-6 has been implicated in numerous chronic inflammatory conditions including obesity, rheumatoid arthritis, and atherosclerosis. Relative to other signaling molecules including TNF-α, IL-6 has a longer half-life and when measured in the circulation is considered a reflection of systemic, low-grade inflammation (Lim & Marsland, 2013). While studies have shown that IL-6 predicts the subsequent risk of all-

cause dementia (Darweesh et al., 2018), we were unable to find any previous work demonstrating that IL-6 predicts conversion from CN to MCI. Based on our results, it is possible that chronically elevated levels of IL-6 may accelerate the deposition of Aβ pathology and, consequently, place individuals at a higher risk of developing cognitive symptoms.

Moderation models in the present study withstood adjustment for a host of factors known to contribute to Aβ pathogenesis, including APOE ε4 status, diabetes, hypertension, history of heart disease, white matter hyperintensities, and depression. Even longitudinal models, which included only half of the original sample size, survived adjustment for white matter pathology, depression, and hypertension, although results were trending after additional adjustment for history of cardiovascular disease. It has previously been suggested that systemic inflammation may be one causal pathway linking cardiovascular disease risk factors to amyloid deposition and AD risk. However, our results indicate that the influence of inflammatory factors on Aβ deposition may reflect a pathway that is mechanistically distinct or independent of the impacts of cardiovascular risk factors. This possibility is supported by epidemiological studies showing that pro-inflammatory conditions without a cardiovascular disease component, such as osteoarthritis (Chen et al., 2018) and periodontitis (Teixeira et al., 2017), are also associated with increased risk of AD. Of note, however, the present study used binary threshold cut-offs to represent cardiovascular risk factors (e.g., diabetes Y/N). The range or magnitude of CVD risk factors may be better represented with continuous variables, which may increase the power and sensitivity to detect relationships between CVD risk factors and inflammatory biomarkers.

4.4 LIMITATIONS

While this large, longitudinal sample of older adults is unique in many ways, certain sample characteristics may limit the generalizability of this study. Specifically, a survival effect is likely to limit interpretation, as the advanced age of this sample (mean age = 86 at baseline imaging) exceeds the average United States lifespan, suggesting that these subjects may be healthier and may have a more adaptive immune system than the general population. Further, the presence of Aβ pathology without corresponding clinical symptoms at this age suggests these subjects may contain protective factors that may not be present to a similar degree in the broader population. However, given that we were able to detect these results in a relatively healthy elderly sample, it's likely that the magnitude of these relationships might be even larger in a general population sample evidencing greater cardiovascular burden and fewer protective factors. There was also a significant loss to follow-up between the 2009 and 2011 imaging assessments, with the sample size decreased by ~ 45%, which further limits interpretation. Of note, however, those that underwent neuroimaging once again in 2011 did not significantly differ demographically or by weight, APOE genotype, or cognitive status from the full baseline sample.

In addition, inflammatory data was only taken at a single time-point and may not be representative of the inflammatory milieu at the time of follow-up imaging. Furthermore, the study sample represents a subset of subjects that had participated in a clinical trial. Therefore, blood data was collected at the close-out visit of the clinical trial and the first PiB scan, considered baseline in the present study, was completed an average of 82 weeks later. Follow-up imaging was collected an average of 185.2 weeks (~ 3.5 years) after initial blood draw. Although other studies have shown that these biomarkers have moderate to high stability over time (particularly IL-6) (Alley et al., 2007; Clendenen et al., 2010; Epstein et al., 2013), multiple assessments would help to

confirm the longitudinal validity of these findings. The lack of multiple assessments of inflammatory biomarkers precludes our ability to make any causal interpretations from our findings. However, given that we observed these relationships both cross-sectionally and longitudinally despite the duration between biomarker collection and neuroimaging, the magnitude of these associations might be even stronger if these variables were more temporally connected. Additionally, the use of statins and anti-inflammatory medications were not controlled for in the present study and may impact the reported associations.

The present study used a TNF-α variable that reflected an average of soluble TNF-α 1 and 2 receptor levels to minimize type 2 error. Evaluating TNF receptor levels is advantageous in longitudinal studies, as solube TNF-α receptors are considered to be more stable over time than TNF-α itself (Lai et al., 2017). However, mouse models indicate potential mechanistic differences between these receptors, with one exhibiting more of a neuroprotective role than the other (Delaby et al., 2015). Although rarely assessed in humans, one study found that both receptors 1 and 2 predicted conversion from MCI to AD (Diniz et al., 2010), suggesting possible mechanistic similarities in humans. However, future work should evaluate the potentially divergent roles of these receptors in humans.

Given the notably smaller sample size containing longitudinal data, we were likely underpowered to detect interaction effects between inflammatory mediators and hippocampal volume on changes in PiB retention over the follow-up periods. Furthermore, the hippocampal atrophy variable reflected hippocampal volume at baseline. While this was appropriate for the cross-sectional analysis, the rate or magnitude of hippocampal change may serve as a more sensitive moderator to explore change in amyloid burden over time. In addition, our moderation analyses may not fully capture individuals that have surpassed the initial preclinical stage of AD.

The presence of significant hippocampal atrophy, hypometabolism, or tauopathy correspond to the second phase of preclinical pathology. The latter two biomarkers were not evaluated in the present study and therefore, we were unable to identify individuals exhibiting these manifestations of preclinical AD pathology. Although one of the most commonly used tracers to identify and quantify Aβ plaque deposition, (PiB) is only capable of detecting insoluble fibrillar Aβ (Heppner et al., 2015). Other forms of Aβ aggregates (Aβ oligomers and monomers) also have pathogenic effects in the brain (Heppner et al., 2015), and therefore results from PET imaging may not completely capture the extent of Aβ pathology in the brain.

We used continuous estimates of PIB retention to maximize power and explore associations that may not be detected using conventional dichotomous cut-offs. However, PiB positivity thresholds were originally developed, in part, to distinguish noise from clinically meaningful tracer binding (Grothe et al., 2017). Therefore, by using continuous measures, we may be uncovering differences in PiB uptake that are not pathologically significant. However, recent epidemiological studies provide support for the behavioral and pathogenic relevance of subthreshold amyloid deposition (Grothe et al., 2017, Landea et al., 2018, Leal et al., 2018). This work has shown that elevated Aβ concentrations in CN older adults classified as amyloid negative (PiB-) using standard thresholds predicts subsequent declines in cognitive performance (Landea et al., 2018) and greater tau deposition 4-5 years later (Leal et al., 2018). These findings underscore the important information that can be derived using continuous estimates of Aβ deposition, particularly among studies interested in the earliest phases of disease onset.

The present study also evaluated associations between inflammatory biomarkers and regional PiB retention. Global indicators are often preferable because of the relatively poor spatial resolution of PET imaging compared to MR imaging, which may limit the precision of the ROIs

(Grothe et al., 2017). Alternatively, regionally specific patterns of Aβ deposition may provide unique information that would otherwise be eclipsed using global amyloid estimates. Indeed, global measures of PiB deposition do not capture the hierarchical progression of amyloid accumulation that takes place in the earliest phases of amyloid pathogenesis (Grothe et al., 2017; Jansen et al., 2018; Villeneuve et al., 2015). As a result, ROI-based approaches have recently been applied to elucidate potential predictors and correlates of early amyloid deposition, including gait speed (Nadkarni et al., 2017), genetic risk factors (Gordon et al., 2018), and obstructive sleep apnea (Yun et al., 2017). While we failed to find a longitudinal relationship between IL-6 and change in global PiB uptake in the present study, ROI analyses enabled us to identify regionally specific associations with frontal and parietal areas that would have been undetected using traditional whole brain estimates.

4.5 FUTURE DIRECTIONS

The pathogenesis of AD is becoming increasingly understood as a complex, multifactorial process that is likely to be affected by factors occurring or originating outside of the brain, including systemic inflammation. It is unlikely that pro-inflammatory processes represent the single molecular event that precipitates the initial generation and aggregation of pathological Aβ peptides in AD. Rather than a precursor, systemic inflammatory may act as an accelerator, hastening an ongoing or underlying neurodegenerative process. Consistent with this, our results suggest that those suffering from low-grade, chronic systemic inflammation may also have a heightened risk of Aβ progression and clinically significant cognitive decline. To confirm and expand upon these results, additional longitudinal studies involving assessments of inflammatory biomarkers at

multiple time points are necessary to further elucidate the impact of systemic inflammation on the nature and rate of disease progression in AD.

Evaluating correlates of Aβ deposition using a more comprehensive panel of inflammatory biomarkers, including chemokines, cell surface receptors, and both pro- and anti-inflammatory cytokines, is needed to more precisely identify protein signatures of preclinical AD. Our results suggest that biomarker concentrations may peak or become particularly relevant during distinct time points of preclinical disease development. For example, we found direct associations between cell-surface receptor CD14 and amyloid deposition, whereas TNF-α and IL-6 associations with amyloid deposition were only revealed when hippocampal atrophy was included as a moderator. Circulating inflammatory biomarkers can be rapidly and easily measured in clinical settings, and further characterization of both the cellular and temporal specificity of these relationships could help to nominate potential targets for early intervention.

Findings from the present study may also inform or apply to other persisting questions regarding the onset and progression of AD. Although the duration of illness is variable, on average, individuals survive for 4 to 8 years after the initial diagnosis of AD. However, AD progresses at different rates among affected individuals and some may live as long as 20 years following the initial diagnosis, although the reasons for this variability are not well understood (Association, 2013, 2014). Similarly, while some individuals with MCI (now known as mild neurocognitive disorder) go on to develop AD and other dementias, others remain stable or even reverse to subclinical levels of impairment. The reasons for this inter-individual variability are poorly understood. Peripheral health factors like systemic inflammation may contribute to this heterogeneity. Our results indicate that systemic inflammation predicted conversion from CN to MCI, as well as accelerated regional amyloid accumulation over 2 years. Similarly, a small-scale

longitudinal study found elevated TNF-α concentrations among subjects that progressed from MCI to dementia relative to those whose MCI status remained stable over the course of 12 months (Diniz et al., 2010). Thus, another important avenue for future work is establishing whether (and to what extent) the systemic inflammatory environment moderates the nature and rate of symptom progression across various stages of disease onset.

There is also a limited understanding of the relationship between central (CSF) and peripherally derived inflammatory biomarkers. Emerging evidence indicates that systemic inflammatory processes may impart additive or even distinct effects on Aβ pathogenesis (Bettcher et al., 2018; Swardfager et al., 2010). Future work estimating inflammatory biomarkers in both the blood and CSF may help to distinguish central versus systemic effects of inflammatory processes on AD. Furthermore, along with Aβ peptides, tau pathology is a hallmark characteristic of AD. Transgenic models of AD in rodents have shown that inflammatory processes promote multiple downstream neurodegenerative changes, including tau pathology. For instance, overexpression of pro-inflammatory cytokines IL-1, IL6, and IL-18 has been shown to enhance the aberrant phosphorylation of tau proteins (Wang et al., 2015). However, similar to Aβ, translational work in humans is sparse, particularly regarding relationships with brain-estimated tau pathology. It's possible that inflammatory pathways may be a viable target to modify ***both*** amyloid and tau pathogenesis, necessitating further evaluation of these relationships with tau and amyloid pathology across disease states. This could provide a clearer understanding of whether systemic management of inflammatory processes may help to prevent or slow the progression of AD-related pathology.

These future directions are encompassed in a recent study protocol published in *BMJ,* which seeks to explore the pathophysiological mechanisms by which central and peripheral

inflammation promotes neuropathology. This year-long prospective study will involve collection of both central and peripheral inflammatory biomarkers, structural imaging, and PET-estimated amyloid deposition and tauopathy in populations with AD, progressive supranuclear palsy, frontotemporal dementia, Lewy body dementia, and MCI (Bevan-Jones et al., 2017). These study objectives not only highlight the potential scope of neuropathological change associated with chronic inflammation, but also illustrate the necessity of evaluating the critical role that systemic inflammation may play in the pathogenesis of a range of neurological conditions.

Finally, strategies to reduce systemic inflammation may extend beyond pharmaceutical treatments and include behavioral interventions, such as physical activity (PA) training. Randomized controlled trials have demonstrated the anti-inflammatory effects of PA in older adults, particularly regarding measures of systemic inflammation including interleukin-6, TNF-α, and C-reactive protein (Manuela Crispim Nascimento et al., 2014; Monteiro-Junior et al., 2018; Santos et al., 2012; Sardeli et al., 2018). Notably, preliminary work indicates that greater engagement in PA is associated with lower levels of amyloid deposition in CN older adulthoods (B. M. Brown et al., 2017; Liang et al., 2010) and those with MCI (Baker et al., 2010). Although speculative, PA may modify Aβ deposition by 1) reducing systemic inflammatory signaling and/or 2) altering the neuroinflammatory processes that potentiate Aβ accumulation. By modifying inflammatory pathways, PA may be a promising approach to reduce the development or progression of neurotoxic Aβ. However, the current evidence base is encumbered by small sample sizes and cross-sectional designs. Large, randomized-controlled exercise intervention trials in older adults, involving multiple assessments of amyloid deposition and biomarker expression, will be imperative to evaluate the potentially protective impact of PA on the pathogenesis of AD.

BIBLIOGRAPHY

Agorastos, A., Hauger, R. L., Barkauskas, D. A., Moeller-Bertram, T., Clopton, P. L., Haji, U., . . . Chrousos, G. P. (2014). Circadian rhythmicity, variability and correlation of interleukin-6 levels in plasma and cerebrospinal fluid of healthy men. *Psychoneuroendocrinology, 44*, 71-82.

Aizenstein, H. J., Nebes, R. D., Saxton, J. A., Price, J. C., Mathis, C. A., Tsopelas, N. D., . . . Houck, P. R. (2008). Frequent amyloid deposition without significant cognitive impairment among the elderly. *Archives of Neurology, 65*(11), 1509-1517.

Alley, D. E., Crimmins, E., Bandeen-Roche, K., Guralnik, J., & Ferrucci, L. (2007). Three-Year Change in Inflammatory Markers in Elderly People and Mortality: The Invecchiare in Chianti Study. *Journal of the American Geriatrics Society, 55*(11), 1801-1807.

Alzheimer's Association, A. (2010). Changing the trajectory of Alzheimer's disease: a national imperative. *Chicago, IL: Alzheimer's Association*.

Aschenbrenner, A. J., Gordon, B. A., Benzinger, T. L., Morris, J. C., & Hassenstab, J. J. (2018). Influence of tau PET, amyloid PET, and hippocampal volume on cognition in Alzheimer disease. *Neurology, 91*(9), e859-e866.

Association, A. s. (2011). New criteria and guidelines for the diagno sis of Alzheimer's disease published for first time in 27 years.

Association, A. s. (2013). 2013 Alzheimer's disease facts and figures. *Alzheimer's & dementia, 9*(2), 208-245.

Association, A. s. (2014). 2014 Alzheimer's disease facts and figures. *Alzheimer's & dementia, 10*(2), e47-e92.

Baker, L. D., Frank, L. L., Foster-Schubert, K., Green, P. S., Wilkinson, C. W., McTiernan, A., . . . Cholerton, B. A. (2010). Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *Archives of Neurology, 67*(1), 71-79.

Balducci, C., Frasca, A., Zotti, M., La Vitola, P., Mhillaj, E., Grigoli, E., . . . Colombo, L. (2017). Toll-like receptor 4-dependent glial cell activation mediates the impairment in memory establishment induced by β-amyloid oligomers in an acute mouse model of Alzheimer's disease. *Brain, behavior, and immunity, 60*, 188-197.

Barnes, D. E., & Yaffe, K. (2011). The projected effect of risk factor reduction on Alzheimer's disease prevalence. *The Lancet Neurology, 10*(9), 819-828.

Bradburn, S., Sarginson, J., & Murgatroyd, C. A. (2018). Association of peripheral interleukin-6 with global cognitive decline in non-demented adults: a meta-analysis of prospective studies. *Frontiers in aging neuroscience, 9*, 438.

Bronzuoli, M. R., Iacomino, A., Steardo, L., & Scuderi, C. (2016). Targeting neuroinflammation in Alzheimer's disease. *Journal of Inflammation Research, 9*, 199.

Brookmeyer, R., Johnson, E., Ziegler-Graham, K., & Arrighi, H. M. (2007). Forecasting the global burden of Alzheimer's disease. *Alzheimer's & dementia, 3*(3), 186-191.

Brosseron, F., Krauthausen, M., Kummer, M., & Heneka, M. T. (2014). Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. *Molecular neurobiology, 50*(2), 534-544.

Brown, B., Peiffer, J., Taddei, K., Lui, J., Laws, S., Gupta, V. B., . . . Burnham, S. (2013). Physical activity and amyloid-β plasma and brain levels: results from the Australian Imaging, Biomarkers and Lifestyle Study of Ageing. *Molecular psychiatry, 18*(8), 875-881.

Brown, B. M., Sohrabi, H. R., Taddei, K., Gardener, S. L., Rainey-Smith, S. R., Peiffer, J. J., . . . Buckles, V. (2017). Habitual exercise levels are associated with cerebral amyloid load in presymptomatic autosomal dominant Alzheimer's disease. *Alzheimer's & dementia, 13*(11), 1197-1206.

Calsolaro, V., & Edison, P. (2016). Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimer's & dementia, 12*(6), 719-732.

Canter, R. G., Penney, J., & Tsai, L.-H. (2016). The road to restoring neural circuits for the treatment of Alzheimer's disease. *Nature, 539*(7628), 187-196.

Capuron, L., & Miller, A. H. (2011). Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacology & therapeutics, 130*(2), 226-238.

Catellier, D. J., Aleksic, N., Folsom, A. R., & Boerwinkle, E. (2008). Atherosclerosis Risk in Communities (ARIC) Carotid MRI flow cytometry study of monocyte and platelet markers: intraindividual variability and reliability. *Clinical chemistry, 54*(8), 1363-1371.

Chen, K. T., Chen, Y. C., Fan, Y. H., Lin, W. X., Lin, W. C., Wang, Y. H., . . . Wei, J. C. C. (2018). Rheumatic diseases are associated with a higher risk of dementia: A nation-wide, population-based, case-control study. *International journal of rheumatic diseases, 21*(2), 373-380.

Chételat, G., La Joie, R., Villain, N., Perrotin, A., de La Sayette, V., Eustache, F., & Vandenberghe, R. (2013). Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. *NeuroImage: Clinical, 2*, 356-365.

Choi, H. J., Byun, M. S., Yi, D., Choe, Y. M., Sohn, B. K., Baek, H. W., . . . Yoon, E. J. (2016). Association Between Serum Triglycerides and Cerebral Amyloidosis in Cognitively Normal Elderly. *The American Journal of Geriatric Psychiatry, 24*(8), 604-612.

Clendenen, T. V., Arslan, A. A., Lokshin, A. E., Idahl, A., Hallmans, G., Koenig, K. L., . . . Zeleniuch-Jacquotte, A. (2010). Temporal reliability of cytokines and growth factors in EDTA plasma. *BMC research notes, 3*(1), 302.

Counts, S. E., Ikonomovic, M. D., Mercado, N., Vega, I. E., & Mufson, E. J. (2016). Biomarkers for the Early Detection and Progression of Alzheimer's Disease. *Neurotherapeutics*, 1-19.

D'Anna, L., Abu-Rumeileh, S., Fabris, M., Pistis, C., Baldi, A., Sanvilli, N., . . . Valente, M. (2017). Serum interleukin-10 levels correlate with cerebrospinal fluid amyloid beta deposition in Alzheimer disease patients. *Neurodegenerative Diseases, 17*(4-5), 227-234.

Dá Mesquita, S., Ferreira, A. C., Sousa, J. C., Correia-Neves, M., Sousa, N., & Marques, F. (2016). Insights on the pathophysiology of Alzheimer's disease: The crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. *Neuroscience & Biobehavioral Reviews, 68*, 547-562.

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature reviews neuroscience, 9*(1), 46.

Darweesh, S. K., Wolters, F. J., Ikram, M. A., de Wolf, F., Bos, D., & Hofman, A. (2018). Inflammatory markers and the risk of dementia and Alzheimer's disease: A meta-analysis. *Alzheimer's & dementia*.

Decourt, B., Lahiri, D., & Sabbagh, M. (2016). Targeting Tumor Necrosis Factor Alpha for Alzheimer's Disease. *Current Alzheimer Research*.

DeKosky, S. T., Williamson, J. D., Fitzpatrick, A. L., Kronmal, R. A., Ives, D. G., Saxton, J. A., . . . Fried, L. P. (2008). Ginkgo biloba for prevention of dementia: a randomized controlled trial. *Jama, 300*(19), 2253-2262.

Delaby, C., Gabelle, A., Blum, D., Schraen-Maschke, S., Moulinier, A., Boulanghien, J., . . . Lehmann, S. (2015). Central nervous system and peripheral inflammatory processes in Alzheimer's disease: biomarker profiling approach. *Frontiers in neurology, 6*, 181.

Dev, S. I., Moore, R. C., Soontornniyomkij, B., Achim, C. L., Jeste, D. V., & Eyler, L. T. (2017). Peripheral inflammation related to lower fMRI activation during a working memory task and resting functional connectivity among older adults: a preliminary study. *International journal of geriatric psychiatry, 32*(3), 341-349.

Diniz, B. S., Teixeira, A. L., Ojopi, E. B., Talib, L. L., Mendonça, V. A., Gattaz, W. F., & Forlenza, O. V. (2010). Higher serum sTNFR1 level predicts conversion from mild cognitive impairment to Alzheimer's disease. *Journal of Alzheimer's Disease, 22*(4), 1305-1311.

Eikelenboom, P., Hoozemans, J. J., Veerhuis, R., van Exel, E., Rozemuller, A. J., & van Gool, W. A. (2012). Whether, when and how chronic inflammation increases the risk of developing late-onset Alzheimer's disease. *Alzheimer's research & therapy, 4*(3), 15.

Epstein, M. M., Breen, E. C., Magpantay, L., Detels, R., Lepone, L., Penugonda, S., . . . Birmann, B. M. (2013). Temporal stability of serum concentrations of cytokines and soluble receptors measured across two years in low-risk HIV-seronegative men. *Cancer Epidemiology and Prevention Biomarkers, 22*(11), 2009-2015.

Fan, Z., Okello, A. A., Brooks, D. J., & Edison, P. (2015). Longitudinal influence of microglial activation and amyloid on neuronal function in Alzheimer's disease. *Brain*, awv288.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry, 18*(6), 499-502.

Galimberti, D., Fenoglio, C., Lovati, C., Venturelli, E., Guidi, I., Corrà, B., . . . Bresolin, N. (2006). Serum MCP-1 levels are increased in mild cognitive impairment and mild Alzheimer's disease. *Neurobiology of aging, 27*(12), 1763-1768.

Galimberti, D., Fenoglio, C., & Scarpini, E. (2008). Inflammation in neurodegenerative disorders: friend or foe? *Current aging science, 1*(1), 30-41.

Glodzik, L., Rusinek, H., Kamer, A., Pirraglia, E., Tsui, W., Mosconi, L., . . . Williams, S. (2016). Effects of vascular risk factors, statins, and antihypertensive drugs on PiB deposition in cognitively normal subjects. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 2*, 95-104.

Gordon, B. A., McCullough, A., Mishra, S., Blazey, T. M., Su, Y., Christensen, J., . . . Morris, J. C. (2018). Cross-sectional and longitudinal atrophy is preferentially associated with tau rather than amyloid β positron emission tomography pathology. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 10*, 245-252.

Griffin, J. M., Kho, D., Graham, E. S., Nicholson, L. F., & O'Carroll, S. J. (2016). Statins inhibit fibrillary β-amyloid induced inflammation in a model of the human blood brain barrier. *PloS one, 11*(6), e0157483.

Grothe, M. J., Barthel, H., Sepulcre, J., Dyrba, M., Sabri, O., Teipel, S. J., . . . Jack, C. R. (2017). In vivo staging of regional amyloid deposition. *Neurology, 89*(20), 2031-2038.

Gu, Y., Zeleniuch-Jacquotte, A., Linkov, F., Koenig, K. L., Liu, M., Velikokhatnaya, L., . . . Lokshin, A. E. (2009). Reproducibility of serum cytokines and growth factors. *Cytokine, 45*(1), 44-49.

Guo, J.-t., Yu, J., Grass, D., de Beer, F. C., & Kindy, M. S. (2002). Inflammation-dependent cerebral deposition of serum amyloid a protein in a mouse model of amyloidosis. *Journal of Neuroscience, 22*(14), 5900-5909.

Gupta, A., & Iadecola, C. (2015). Impaired Aβ clearance: a potential link between atherosclerosis and Alzheimer's disease. *Frontiers in aging neuroscience, 7*.

Harada, R., Ishiki, A., Kai, H., Sato, N., Furukawa, K., Furumoto, S., . . . Hiraoka, K. (2018). Correlations of 18F-THK5351 PET with postmortem burden of tau and astrogliosis in Alzheimer disease. *Journal of Nuclear Medicine, 59*(4), 671-674.

Hardikar, S., Song, X., Kratz, M., Anderson, G. L., Blount, P. L., Reid, B. J., . . . White, E. (2014). Intraindividual variability over time in plasma biomarkers of inflammation and effects of long-term storage. *Cancer Causes & Control, 25*(8), 969-976.

He, P., Cheng, X., Staufenbiel, M., Li, R., & Shen, Y. (2013). Long-term treatment of thalidomide ameliorates amyloid-like pathology through inhibition of β-secretase in a mouse model of Alzheimer's disease. *PloS one, 8*(2), e55091.

Head, D., Bugg, J. M., Goate, A. M., Fagan, A. M., Mintun, M. A., Benzinger, T., . . . Morris, J. C. (2012). Exercise engagement as a moderator of the effects of APOE genotype on amyloid deposition. *Archives of Neurology, 69*(5), 636-643.

Hedden, T., Oh, H., Younger, A. P., & Patel, T. A. (2013). Meta-analysis of amyloid-cognition relations in cognitively normal older adults. *Neurology, 80*(14), 1341-1348.

Heneka, M. T., Carson, M. J., El Khoury, J., Landreth, G. E., Brosseron, F., Feinstein, D. L., . . . Ransohoff, R. M. (2015). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology, 14*(4), 388-405.

Heppner, F. L., Ransohoff, R. M., & Becher, B. (2015). Immune attack: the role of inflammation in Alzheimer disease. *Nature reviews neuroscience, 16*(6), 358-372.

Heringa, S. M., van den Berg, E., Reijmer, Y. D., Nijpels, G., Stehouwer, C. D., Schalkwijk, C. G., . . . Kappelle, L. J. (2014). Markers of low-grade inflammation and endothelial dysfunction are related to reduced information processing speed and executive functioning in an older population–the Hoorn study. *Psychoneuroendocrinology, 40*, 108-118.

Hickman, S. E., Allison, E. K., & El Khoury, J. (2008). Microglial dysfunction and defective β-amyloid clearance pathways in aging Alzheimer's disease mice. *Journal of Neuroscience, 28*(33), 8354-8360.

Holmes, C., Cunningham, C., Zotova, E., Culliford, D., & Perry, V. (2011). Proinflammatory cytokines, sickness behavior, and Alzheimer disease. *Neurology*, WNL. 0b013e318225ae318207.

Holmes, C., Cunningham, C., Zotova, E., Woolford, J., Dean, C., Kerr, S. u., . . . Perry, V. (2009). Systemic inflammation and disease progression in Alzheimer disease. *Neurology, 73*(10), 768-774.

Hughes, T. M., Kuller, L. H., Barinas-Mitchell, E. J., McDade, E. M., Klunk, W. E., Cohen, A. D., . . . Lopez, O. L. (2014). Arterial stiffness and β-amyloid progression in nondemented elderly adults. *JAMA neurology, 71*(5), 562-568.

Hughes, T. M., Lopez, O. L., Evans, R. W., Kamboh, M. I., Williamson, J. D., Klunk, W. E., . . . Snitz, B. E. (2014). Markers of cholesterol transport are associated with amyloid deposition in the brain. *Neurobiology of aging, 35*(4), 802-807.

In'T Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., . . . Stricker, B. H. (2001). Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. *New England Journal of Medicine, 345*(21), 1515-1521.

Insel, P. S., Mattsson, N., Mackin, R. S., Schöll, M., Nosheny, R. L., Tosun, D., . . . Weiner, M. W. (2016). Accelerating rates of cognitive decline and imaging markers associated with β-amyloid pathology. *Neurology, 86*(20), 1887-1896.

Jack, C. R., Albert, M. S., Knopman, D. S., McKhann, G. M., Sperling, R. A., Carrillo, M. C., . . . Phelps, C. H. (2011). Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia, 7*(3), 257-262.

Jack, C. R., Bernstein, M. A., Fox, N. C., Thompson, P., Alexander, G., Harvey, D., . . . Ward, C. (2008). The Alzheimer's disease neuroimaging initiative (ADNI): MRI methods. *Journal of magnetic resonance imaging, 27*(4), 685-691.

Jack, C. R., Wiste, H. J., Knopman, D. S., Vemuri, P., Mielke, M. M., Weigand, S. D., . . . Gregg, B. E. (2014). Rates of β-amyloid accumulation are independent of hippocampal neurodegeneration. *Neurology, 82*(18), 1605-1612.

Jackson, S. E., Van Jaarsveld, C. H., Beeken, R. J., Gunter, M. J., Steptoe, A., & Wardle, J. (2015). Four-year stability of anthropometric and cardio-metabolic parameters in a prospective cohort of older adults. *Biomarkers, 9*(2), 109-122.

Jacobs, H. I., Hedden, T., Schultz, A. P., Sepulcre, J., Perea, R. D., Amariglio, R. E., . . . Johnson, K. A. (2018). Structural tract alterations predict downstream tau accumulation in amyloid-positive older individuals. *Nature neuroscience, 21*(3), 424.

Jagust, W. J., Bandy, D., Chen, K., Foster, N. L., Landau, S. M., Mathis, C. A., . . . Koeppe, R. A. (2010). The Alzheimer's Disease Neuroimaging Initiative positron emission tomography core. *Alzheimer's & dementia, 6*(3), 221-229.

Janelidze, S., Mattsson, N., Stomrud, E., Lindberg, O., Palmqvist, S., Zetterberg, H., . . . Hansson, O. (2018). CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease. *Neurology, 91*(9), e867-e877.

Jansen, W. J., Ossenkoppele, R., Knol, D. L., Tijms, B. M., Scheltens, P., Verhey, F. R., . . . Alcolea, D. (2015). Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. *Jama, 313*(19), 1924-1938.

Jansen, W. J., Ossenkoppele, R., Tijms, B. M., Fagan, A. M., Hansson, O., Klunk, W. E., . . . Fleisher, A. S. (2018). Association of cerebral amyloid-β aggregation with cognitive functioning in persons without dementia. *JAMA psychiatry, 75*(1), 84-95.

Kantarci, K., Lowe, V., Przybelski, S., Weigand, S., Senjem, M., Ivnik, R. J., . . . Boeve, B. F. (2012). APOE modifies the association between Aβ load and cognition in cognitively normal older adults. *Neurology, 78*(4), 232-240.

Karran, E., Mercken, M., & De Strooper, B. (2011). The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nature reviews Drug discovery, 10*(9), 698-712.

Kim, S.-M., Kim, B.-Y., Eo, S.-K., Kim, C.-D., & Kim, K. (2015). 27-Hydroxycholesterol up-regulates CD14 and predisposes monocytic cells to superproduction of CCL2 in response to lipopolysaccharide. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1852*(3), 442-450.

Kivipelto, M., Helkala, E.-L., Laakso, M. P., Hänninen, T., Hallikainen, M., Alhainen, K., . . . Nissinen, A. (2001). Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *Bmj, 322*(7300), 1447-1451.

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., . . . Nissinen, A. (2005). Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Archives of Neurology, 62*(10), 1556-1560.

Knopman, D. S., Jack, C., Wiste, H., Weigand, S., Vemuri, P., Lowe, V., . . . Ivnik, R. J. (2012). Short-term clinical outcomes for stages of NIA-AA preclinical Alzheimer disease. *Neurology, 78*(20), 1576-1582.

Krstic, D., & Knuesel, I. (2013). Deciphering the mechanism underlying late-onset Alzheimer disease. *Nature Reviews Neurology, 9*(1), 25-34.

Kyrkanides, S., Tallents, R. H., Jen-nie, H. M., Olschowka, M. E., Johnson, R., Yang, M., . . . O'Banion, M. K. (2011). Osteoarthritis accelerates and exacerbates Alzheimer's disease pathology in mice. *Journal of Neuroinflammation, 8*(1), 112.

Lai, K. S. P., Liu, C. S., Rau, A., Lanctôt, K. L., Köhler, C. A., Pakosh, M., . . . Herrmann, N. (2017). Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. *J Neurol Neurosurg Psychiatry*, jnnp-2017-316201.

Leung, E., Guo, L., Bu, J., Maloof, M., El Khoury, J., & Geula, C. (2011). Microglia activation mediates fibrillar amyloid-β toxicity in the aged primate cortex. *Neurobiology of aging, 32*(3), 387-397.

Liang, K. Y., Mintun, M. A., Fagan, A. M., Goate, A. M., Bugg, J. M., Holtzman, D. M., . . . Head, D. (2010). Exercise and Alzheimer's disease biomarkers in cognitively normal older adults. *Annals of neurology, 68*(3), 311-318.

Liao, Y.-F., Wang, B.-J., Cheng, H.-T., Kuo, L.-H., & Wolfe, M. S. (2004). Tumor necrosis factor-α, interleukin-1β, and interferon-γ stimulate γ-secretase-mediated cleavage of amyloid precursor protein through a JNK-dependent MAPK pathway. *Journal of Biological Chemistry, 279*(47), 49523-49532.

Lim, A., Krajina, K., & Marsland, A. L. (2013). Peripheral inflammation and cognitive aging. Mod Trends Pharmacopsychiatry 28: 175–187.

Lim, Y. Y., Ellis, K. A., Ames, D., Darby, D., Harrington, K., Martins, R. N., . . . Szoeke, C. (2013). Aβ amyloid, cognition, and APOE genotype in healthy older adults. *Alzheimer's & dementia, 9*(5), 538-545.

Lim, Y. Y., Maruff, P., Pietrzak, R. H., Ames, D., Ellis, K. A., Harrington, K., . . . Masters, C. L. (2013). Effect of amyloid on memory and non-memory decline from preclinical to clinical Alzheimer's disease. *Brain*, awt286.

Lindqvist, D., Janelidze, S., Hagell, P., Erhardt, S., Samuelsson, M., Minthon, L., . . . Brundin, L. (2009). Interleukin-6 is elevated in the cerebrospinal fluid of suicide attempters and related to symptom severity. *Biological psychiatry, 66*(3), 287-292.

Liu, X., Yu, Y., & Zhu, S. (2018). Inflammatory markers in postoperative delirium (POD) and cognitive dysfunction (POCD): A meta-analysis of observational studies. *PloS one, 13*(4), e0195659.

Lockhart, S. N., Schöll, M., Baker, S. L., Ayakta, N., Swinnerton, K. N., Bell, R. K., . . . Janabi, M. (2017). Amyloid and tau PET demonstrate region-specific associations in normal older people. *Neuroimage, 150*, 191-199.

Lopez, O. L., Klunk, W. E., Mathis, C., Coleman, R. L., Price, J., Becker, J. T., . . . Ikonomovic, M. (2014). Amyloid, neurodegeneration, and small vessel disease as predictors of dementia in the oldest-old. *Neurology, 83*(20), 1804-1811.

Macchi, B., Marino-Merlo, F., Nocentini, U., Pisani, V., Cuzzocrea, S., Grelli, S., & Mastino, A. (2015). Role of inflammation and apoptosis in multiple sclerosis: Comparative analysis between the periphery and the central nervous system. *Journal of neuroimmunology, 287*, 80-87.

MacPherson, K. P., Sompol, P., Kannarkat, G. T., Chang, J., Sniffen, L., Wildner, M. E., . . . Tansey, M. G. (2017). Peripheral administration of the soluble TNF inhibitor XPro1595 modifies brain immune cell profiles, decreases beta-amyloid plaque load, and rescues impaired long-term potentiation in 5xFAD mice. *Neurobiology of disease, 102*, 81-95.

Maes, M., Berk, M., Goehler, L., Song, C., Anderson, G., Gałecki, P., & Leonard, B. (2012). Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC medicine, 10*(1), 66.

Magalhães, T., Weiler, M., Teixeira, C., Hayata, T., Moraes, A., Boldrini, V., . . . Joaquim, H. (2018). Systemic inflammation and multimodal biomarkers in amnestic mild cognitive impairment and Alzheimer's disease. *Molecular neurobiology, 55*(7), 5689-5697.

Manuela Crispim Nascimento, C., Rodrigues Pereira, J., Pires de Andrade, L., Garuffi, M., Leme Talib, L., Vicente Forlenza, O., . . . Stella, F. (2014). Physical exercise in MCI elderly promotes reduction of pro-inflammatory cytokines and improvements on cognition and BDNF peripheral levels. *Current Alzheimer Research, 11*(8), 799-805.

Marchant, N. L., Reed, B. R., Sanossian, N., Madison, C. M., Kriger, S., Dhada, R., . . . Mungas, D. M. (2013). The aging brain and cognition: contribution of vascular injury and aβ to mild cognitive dysfunction. *JAMA neurology, 70*(4), 488-495.

Marsland, A. L., Gianaros, P. J., Kuan, D. C.-H., Sheu, L. K., Krajina, K., & Manuck, S. B. (2015). Brain morphology links systemic inflammation to cognitive function in midlife adults. *Brain, behavior, and immunity, 48*, 195-204.

Marsland, A. L., Kuan, D. C.-H., Sheu, L. K., Krajina, K., Kraynak, T. E., Manuck, S. B., & Gianaros, P. J. (2017). Systemic inflammation and resting state connectivity of the default mode network. *Brain, behavior, and immunity, 62*, 162-170.

Mathis, C. A., Kuller, L. H., Klunk, W. E., Snitz, B. E., Price, J. C., Weissfeld, L. A., . . . Aizenstein, H. J. (2013). In vivo assessment of amyloid-β deposition in nondemented very elderly subjects. *Annals of neurology, 73*(6), 751-761.

McKay, H. S., Margolick, J. B., Martínez-Maza, O., Lopez, J., Phair, J., Rappocciolo, G., . . . Bream, J. H. (2017). Multiplex assay reliability and long-term intra-individual variation of serologic inflammatory biomarkers. *Cytokine, 90*, 185-192.

Metti, A. L., Aizenstein, H., Yaffe, K., Boudreau, R. M., Newman, A., Launer, L., . . . Ives, D. G. (2015). Trajectories of peripheral interleukin-6, structure of the hippocampus, and cognitive impairment over 14 years in older adults. *Neurobiology of aging, 36*(11), 3038-3044.

Mietelska-Porowska, A., & Wojda, U. (2017). T lymphocytes and inflammatory mediators in the interplay between brain and blood in Alzheimer's disease: potential pools of new biomarkers. *Journal of immunology research, 2017*.

Miwa, K., Tanaka, M., Okazaki, S., Furukado, S., Sakaguchi, M., & Kitagawa, K. (2011). Relations of blood inflammatory marker levels with cerebral microbleeds. *Stroke, 42*(11), 3202-3206.

Monteiro-Junior, R. S., de Tarso Maciel-Pinheiro, P., da Matta Mello Portugal, E., da Silva Figueiredo, L. F., Terra, R., Carneiro, L. S., . . . Laks, J. (2018). Effect of exercise on inflammatory profile of older persons: systematic review and meta-analyses. *Journal of Physical Activity and Health, 15*(1), 64-71.

Mormino, E. C., Betensky, R. A., Hedden, T., Schultz, A. P., Amariglio, R. E., Rentz, D. M., . . . Sperling, R. A. (2014). Synergistic effect of β-amyloid and neurodegeneration on cognitive decline in clinically normal individuals. *JAMA neurology, 71*(11), 1379-1385.

Mortamais, M., Ash, J. A., Harrison, J., Kaye, J., Kramer, J., Randolph, C., . . . Ritchie, C. W. (2016). Detecting cognitive changes in preclinical Alzheimer's disease: A review of its feasibility. *Alzheimer's & dementia*.

Murphy, M. P., & LeVine III, H. (2010). Alzheimer's disease and the amyloid-β peptide. *Journal of Alzheimer's Disease, 19*(1), 311-323.

Musiek, E. S., & Holtzman, D. M. (2015). Three dimensions of the amyloid hypothesis: time, space and'wingmen'. *Nature neuroscience*, 800-806.

Nadkarni, N. K., Perera, S., Snitz, B. E., Mathis, C. A., Price, J., Williamson, J. D., . . . Lopez, O. L. (2017). Association of brain amyloid-β with slow gait in elderly individuals without dementia: influence of cognition and apolipoprotein E ε4 genotype. *JAMA neurology, 74*(1), 82-90.

Nash, S. D., Cruickshanks, K. J., Klein, R., Klein, B. E., Javier Nieto, F., Chappell, R., . . . Tsai, M. Y. (2013). Long-term variability of inflammatory markers and associated factors in a population-based cohort. *Journal of the American Geriatrics Society, 61*(8), 1269-1276.

Navarro, S. L., Brasky, T. M., Schwarz, Y., Song, X., Wang, C., Kristal, A. R., . . . Lampe, J. W. (2012). Reliability of serum biomarkers of inflammation from repeated measures in healthy individuals. *Cancer Epidemiology and Prevention Biomarkers, 21*(7), 1167-1170.

Oh, H., Madison, C., Villeneuve, S., Markley, C., & Jagust, W. J. (2013). Association of gray matter atrophy with age, β-amyloid, and cognition in aging. *Cerebral Cortex*, bht017.

Out, D., Hall, R. J., Granger, D. A., Page, G. G., & Woods, S. J. (2012). Assessing salivary C-reactive protein: longitudinal associations with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. *Brain, behavior, and immunity, 26*(4), 543-551.

Palta, P., Xue, Q.-L., Deal, J. A., Fried, L. P., Walston, J. D., & Carlson, M. C. (2014). Interleukin-6 and C-reactive protein levels and 9-year cognitive decline in community-dwelling older women: the women's health and aging study II. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 70*(7), 873-878.

Parbo, P., Ismail, R., Sommerauer, M., Stokholm, M. G., Hansen, A. K., Hansen, K. V., . . . Brændgaard, H. (2018). Does inflammation precede tau aggregation in early Alzheimer's disease? A PET study. *Neurobiology of disease*.

Petersen, R. C., Wiste, H. J., Weigand, S. D., Rocca, W. A., Roberts, R. O., Mielke, M. M., . . . Machulda, M. M. (2016). Association of elevated amyloid levels with cognition and biomarkers in cognitively normal people from the community. *JAMA neurology, 73*(1), 85-92.

Prestia, A., Caroli, A., Van Der Flier, W. M., Ossenkoppele, R., Van Berckel, B., Barkhof, F., . . . Schöll, M. (2013). Prediction of dementia in MCI patients based on core diagnostic markers for Alzheimer disease. *Neurology, 80*(11), 1048-1056.

Price, J. C., Klunk, W. E., Lopresti, B. J., Lu, X., Hoge, J. A., Ziolko, S. K., . . . Mathis, C. A. (2005). Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh Compound-B. *Journal of Cerebral Blood Flow & Metabolism, 25*(11), 1528-1547.

Racine, A. M., Koscik, R. L., Nicholas, C. R., Clark, L. R., Okonkwo, O. C., Oh, J. M., . . . Betthauser, T. J. (2016). Cerebrospinal fluid ratios with Aβ 42 predict preclinical brain β-amyloid accumulation. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 2*, 27-38.

Ramesh, G., MacLean, A. G., & Philipp, M. T. (2013). Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. *Mediators of inflammation, 2013*.

Ramlackhansingh, A. F., Brooks, D. J., Greenwood, R. J., Bose, S. K., Turkheimer, F. E., Kinnunen, K. M., . . . Gelosa, G. (2011). Inflammation after trauma: microglial activation and traumatic brain injury. *Annals of neurology, 70*(3), 374-383.

Rentz, D. M., Locascio, J. J., Becker, J. A., Moran, E. K., Eng, E., Buckner, R. L., . . . Johnson, K. A. (2010). Cognition, reserve, and amyloid deposition in normal aging. *Annals of neurology, 67*(3), 353-364.

Rethorst, C. D., Bernstein, I., & Trivedi, M. H. (2014). Inflammation, obesity and metabolic syndrome in depression: Analysis of the 2009–2010 National Health and Nutrition Survey (NHANES). *The Journal of clinical psychiatry, 75*(12), e1428.

Rosario, B. L., Weissfeld, L. A., Laymon, C. M., Mathis, C. A., Klunk, W. E., Berginc, M. D., . . . Price, J. C. (2011). Inter-rater reliability of manual and automated region-of-interest delineation for PiB PET. *Neuroimage, 55*(3), 933-941.

Rubio-Perez, J. M., & Morillas-Ruiz, J. M. (2012). A review: inflammatory process in Alzheimer's disease, role of cytokines. *The Scientific World Journal, 2012*.

Saleem, M., Herrmann, N., Swardfager, W., Eisen, R., & Lanctot, K. L. (2015). Inflammatory markers in mild cognitive impairment: a meta-analysis. *Journal of Alzheimer's Disease, 47*(3), 669-679.

Santillo, A. F., Gambini, J. P., Lannfelt, L., Långström, B., Ulla-Marja, L., Kilander, L., & Engler, H. (2011). In vivo imaging of astrocytosis in Alzheimer's disease: an 11C-L-deuteriodeprenyl and PIB PET study. *European journal of nuclear medicine and molecular imaging, 38*(12), 2202-2208.

Santos, R. d., Viana, V. A. R., Boscolo, R. A., Marques, V., Santana, M. G. d., Lira, F. S. d., . . . De Mello, M. (2012). Moderate exercise training modulates cytokine profile and sleep in elderly people. *Cytokine, 60*(3), 731-735.

Sardeli, A. V., Tomeleri, C. M., Cyrino, E. S., Fernhall, B., Cavaglieri, C. R., & Chacon-Mikahil, M. P. T. (2018). Effect of resistance training on inflammatory markers of older adults: A meta-analysis. *Experimental gerontology*.

Sasayama, D., Hattori, K., Wakabayashi, C., Teraishi, T., Hori, H., Ota, M., . . . Amano, N. (2013). Increased cerebrospinal fluid interleukin-6 levels in patients with schizophrenia and those with major depressive disorder. *Journal of psychiatric research, 47*(3), 401-406.

Schuitemaker, A., Kropholler, M. A., Boellaard, R., van der Flier, W. M., Kloet, R. W., van der Doef, T. F., . . . Barkhof, F. (2013). Microglial activation in Alzheimer's disease: an (R)-[11 C] PK11195 positron emission tomography study. *Neurobiology of aging, 34*(1), 128-136.

Schultz, S. A., Boots, E. A., Darst, B. F., Zetterberg, H., Blennow, K., Edwards, D. F., . . . Bendlin, B. B. (2017). Cardiorespiratory fitness alters the influence of a polygenic risk score on biomarkers of AD. *Neurology, 88*(17), 1650-1658.

Shamim, D., & Laskowski, M. (2017). Inhibition of inflammation mediated through the tumor necrosis factor α biochemical pathway can lead to favorable outcomes in Alzheimer disease. *Journal of central nervous system disease, 9*, 1179573517722512.

Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., . . . Lemere, C. A. (2008). Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature medicine, 14*(8), 837-842.

Sharma, N., & Singh, A. N. (2016). Exploring Biomarkers for Alzheimer's Disease. *Journal of Clinical and Diagnostic Research: JCDR, 10*(7), KE01.

Sheng, J. G., Bora, S. H., Xu, G., Borchelt, D. R., Price, D. L., & Koliatsos, V. E. (2003). Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid β peptide in APPswe transgenic mice. *Neurobiology of disease, 14*(1), 133-145.

Simone, M. J., & Tan, Z. S. (2011). The role of inflammation in the pathogenesis of delirium and dementia in older adults: a review. *CNS neuroscience & therapeutics, 17*(5), 506-513.

Snitz, B. E., O'meara, E. S., Carlson, M. C., Arnold, A. M., Ives, D. G., Rapp, S. R., . . . Sink, K. M. (2009). Ginkgo biloba for preventing cognitive decline in older adults: a randomized trial. *Jama, 302*(24), 2663-2670.

Snitz, B. E., Weissfeld, L. A., Lopez, O. L., Kuller, L. H., Saxton, J., Singhabahu, D. M., . . . Ives, D. G. (2013). Cognitive trajectories associated with β-amyloid deposition in the oldest-old without dementia. *Neurology, 80*(15), 1378-1384.

Sone, D., Imabayashi, E., Maikusa, N., Okamura, N., Furumoto, S., Kudo, Y., . . . Sakata, M. (2017). Regional tau deposition and subregion atrophy of medial temporal structures in early Alzheimer's disease: A combined positron emission tomography/magnetic resonance

imaging study. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 9*, 35-40.

Sperling, R., Mormino, E., & Johnson, K. (2014). The evolution of preclinical Alzheimer's disease: implications for prevention trials. *Neuron, 84*(3), 608-622.

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., . . . Montine, T. J. (2011). Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia, 7*(3), 280-292.

Sperling, R. A., Karlawish, J., & Johnson, K. A. (2013). Preclinical Alzheimer disease—the challenges ahead. *Nature Reviews Neurology, 9*(1), 54-58.

Srikanthan, K., Feyh, A., Visweshwar, H., Shapiro, J. I., & Sodhi, K. (2016). Systematic review of metabolic syndrome biomarkers: a panel for early detection, management, and risk stratification in the West Virginian population. *International journal of medical sciences, 13*(1), 25.

Stenfors, C. U., Jonsdottir, I., Hanson, L. M., & Theorell, T. (2017). Associations between systemic pro-inflammatory markers, cognitive function and cognitive complaints in a population-based sample of working adults. *Journal of psychosomatic research, 96*, 49-59.

Storandt, M., Mintun, M. A., Head, D., & Morris, J. C. (2009). Cognitive decline and brain volume loss as signatures of cerebral amyloid-β peptide deposition identified with Pittsburgh compound B: cognitive decline associated with Aβ deposition. *Archives of Neurology, 66*(12), 1476-1481.

Strauss, S., Bauer, J., Ganter, U., Jonas, U., Berger, M., & Volk, B. (1992). Detection of interleukin-6 and alpha 2-macroglobulin immunoreactivity in cortex and hippocampus of Alzheimer's disease patients. *Laboratory investigation; a journal of technical methods and pathology, 66*(2), 223-230.

Swardfager, W., Lanctôt, K., Rothenburg, L., Wong, A., Cappell, J., & Herrmann, N. (2010). A meta-analysis of cytokines in Alzheimer's disease. *Biological psychiatry, 68*(10), 930-941.

Tarawneh, R., & Holtzman, D. M. (2012). The clinical problem of symptomatic Alzheimer disease and mild cognitive impairment. *Cold Spring Harbor perspectives in medicine, 2*(5), a006148.

Tarkowski, E., Andreasen, N., Tarkowski, A., & Blennow, K. (2003). Intrathecal inflammation precedes development of Alzheimer's disease. *Journal of Neurology, Neurosurgery & Psychiatry, 74*(9), 1200-1205.

Tegeler, C., O'Sullivan, J. L., Bucholtz, N., Goldeck, D., Pawelec, G., Steinhagen-Thiessen, E., & Demuth, I. (2016). The inflammatory markers CRP, IL-6, and IL-10 are associated with

cognitive function—data from the Berlin Aging Study II. *Neurobiology of aging, 38*, 112-117.

Teixeira, F. B., Saito, M. T., Matheus, F. C., Prediger, R. D., Yamada, E. S., Maia, C. S., & Lima, R. R. (2017). Periodontitis and Alzheimer's Disease: A Possible Comorbidity between Oral Chronic Inflammatory Condition and Neuroinflammation. *Frontiers in aging neuroscience, 9*, 327.

Togo, T., Akiyama, H., Iseki, E., Kondo, H., Ikeda, K., Kato, M., . . . Kosaka, K. (2002). Occurrence of T cells in the brain of Alzheimer's disease and other neurological diseases. *Journal of neuroimmunology, 124*(1-2), 83-92.

Tsirpanlis, G., Boufidou, F., Zoga, M., Triantafyllis, G., Fatourou, A., Stamatelou, K., . . . Nicolaou, C. (2009). Low cholesterol along with inflammation predicts morbidity and mortality in hemodialysis patients. *Hemodialysis International, 13*(2), 197-204.

Tweedie, D., Ferguson, R. A., Fishman, K., Frankola, K. A., Van Praag, H., Holloway, H. W., . . . Russo, I. (2012). Tumor necrosis factor-α synthesis inhibitor 3, 6′-dithiothalidomide attenuates markers of inflammation, Alzheimer pathology and behavioral deficits in animal models of neuroinflammation and Alzheimer's disease. *Journal of Neuroinflammation, 9*(1), 1.

Villemagne, V. L., Pike, K. E., Chételat, G., Ellis, K. A., Mulligan, R. S., Bourgeat, P., . . . Salvado, O. (2011). Longitudinal assessment of Aβ and cognition in aging and Alzheimer disease. *Annals of neurology, 69*(1), 181-192.

Villeneuve, S., & Jagust, W. J. (2015). Imaging vascular disease and amyloid in the aging brain: implications for treatment. *The journal of prevention of Alzheimer's disease, 2*(1), 64.

Villeneuve, S., Rabinovici, G. D., Cohn-Sheehy, B. I., Madison, C., Ayakta, N., Ghosh, P. M., . . . Marks, S. M. (2015). Existing Pittsburgh Compound-B positron emission tomography thresholds are too high: statistical and pathological evaluation. *Brain, 138*(7), 2020-2033.

Vlassenko, A. G., Benzinger, T. L., & Morris, J. C. (2012). PET amyloid-beta imaging in preclinical Alzheimer's disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1822*(3), 370-379.

Vos, S. J., Xiong, C., Visser, P. J., Jasielec, M. S., Hassenstab, J., Grant, E. A., . . . Fagan, A. M. (2013). Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. *The Lancet Neurology, 12*(10), 957-965.

Walters, A., Phillips, E., Zheng, R., Biju, M., & Kuruvilla, T. (2016). Evidence for neuroinflammation in Alzheimer's disease. *Progress in Neurology and Psychiatry, 20*(5), 25-31.

Wang, E., Zhu, H., Wang, X., Gower, A., Wallack, M., Blusztajn, J. K., . . . Qiu, W. Q. (2016). Amylin Treatment Reduces Neuroinflammation and Ameliorates Abnormal Patterns of

Gene Expression in the Cerebral Cortex of an Alzheimer's Disease Mouse Model. *Journal of Alzheimer's Disease*(Preprint), 1-15.

Wang, J., Gu, B. J., Masters, C. L., & Wang, Y.-J. (2017). A systemic view of Alzheimer disease—insights from amyloid-β metabolism beyond the brain. *Nature Reviews Neurology, 13*(10), 612.

Wang, W.-Y., Tan, M.-S., Yu, J.-T., & Tan, L. (2015). Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Annals of translational medicine, 3*(10).

Warren, K. N., Beason-Held, L. L., Carlson, O., Egan, J. M., An, Y., Doshi, J., . . . Resnick, S. M. (2018). Elevated Markers of Inflammation Are Associated With Longitudinal Changes in Brain Function in Older Adults. *The Journals of Gerontology: Series A, 73*(6), 770-778.

Welty, F. K., Alfaddagh, A., & Elajami, T. K. (2016). Targeting inflammation in metabolic syndrome. *Translational Research, 167*(1), 257-280.

Whitmer, R., Sidney, S., Selby, J., Johnston, S. C., & Yaffe, K. (2005). Midlife cardiovascular risk factors and risk of dementia in late life. *Neurology, 64*(2), 277-281.

Wiley, C. A., Lopresti, B. J., Venneti, S., Price, J., Klunk, W. E., DeKosky, S. T., & Mathis, C. A. (2009). Carbon 11–Labeled Pittsburgh Compound B and Carbon 11–Labeled (R)-PK11195 Positron Emission Tomographic Imaging in Alzheimer Disease. *Archives of Neurology, 66*(1), 60-67.

Wirth, M., Madison, C. M., Rabinovici, G. D., Oh, H., Landau, S. M., & Jagust, W. J. (2013). Alzheimer's disease neurodegenerative biomarkers are associated with decreased cognitive function but not β-amyloid in cognitively normal older individuals. *Journal of Neuroscience, 33*(13), 5553-5563.

Woodcock, T., & Morganti-Kossmann, M. C. (2013). The role of markers of inflammation in traumatic brain injury.

Yan, Q., Zhang, J., Liu, H., Babu-Khan, S., Vassar, R., Biere, A. L., . . . Landreth, G. (2003). Anti-inflammatory drug therapy alters β-amyloid processing and deposition in an animal model of Alzheimer's disease. *Journal of Neuroscience, 23*(20), 7504-7509.

Yasuno, F., Kosaka, J., Ota, M., Higuchi, M., Ito, H., Fujimura, Y., . . . Asada, T. (2012). Increased binding of peripheral benzodiazepine receptor in mild cognitive impairment–dementia converters measured by positron emission tomography with [11 C] DAA1106. *Psychiatry Research: Neuroimaging, 203*(1), 67-74.

Yun, C.-H., Lee, H.-Y., Lee, S. K., Kim, H., Seo, H. S., Bang, S., . . . Shin, C. (2017). Amyloid burden in obstructive sleep apnea. *Journal of Alzheimer's Disease, 59*(1), 21-29.

Zammit, A. R., Katz, M. J., Derby, C., Bitzer, M., & Lipton, R. B. (2015). Chronic kidney disease in non-diabetic older adults: associated roles of the metabolic syndrome, inflammation, and insulin resistance. *PloS one, 10*(10), e0139369.

Zhang, H., Sachdev, P. S., Wen, W., Crawford, J. D., Brodaty, H., Baune, B. T., . . . Kang, K. (2016). The relationship between inflammatory markers and voxel-based gray matter volumes in nondemented older adults. *Neurobiology of aging, 37*, 138-146.